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

Kristina Arranz^a, Iñaki Urrutxurtu^a, Irrintzi Ibarrola^a, Miren Bego Urrutia^a, Carlos Saavedra^b, David Cordero^b, Josu Pérez-Larruscain^c, Enrique Navarro^a

^aDepartamento de Genética, Antropología Física y Fisiología Animal, Facultad de Ciencia y Tecnología, Universidad del País Vasco/Euskal Herriko Unibertsitatea, UPV/EHU, Apartado 644, 48080 Bilbao, Spain

^bInstituto de Acuicultura Torre de la Sal, Consejo Superior de Investigaciones Científicas, 12595 Ribera de Cabanes (Castellón), Spain

^cCentre d'Aquicultura, Institut de Recerca i Tecnologies Agroalimentaries. Crta. Poble Nou, Km 5,5, 43540 Sant Carles de la Ràpita (Tarragona), Spain

Abstract:

A range of phenotypes differing in growth rate were designed in the Manila clam by combining separate breeding families with size segregation within each family to constitute fast and slow growing groups. Physiological components of the energy budget and scope for growth (SFG) were then compared between these different phenotypes during the acute and chronic responses to two diets that were iso-caloric but differed by 3-fold in their protein/energy (P/E) ratios. Both diets were based on the microalgae *Rhodomonas lens* obtained in either the exponential or the stationary phase of culture. The aims of the study were 1) to test the effects of these changes in food composition on growth rate, estimated as

the balance of physiological processes of energy gain and loss integrated in the SFG; and 2) to assess the extent to which physiological adjustments to diet composition are modulated in order to fulfill the variable energy requirements posed by the occurrence of differential growth phenotypes. Growth performance improved with the high-protein (N+) diet for the different family * growth group combinations, with SFG values exceeding by 50% on average the values of the low-protein (N-) diet. Digestive constraints resulted in reduced absorption efficiency with the N-diet, which tended to cancel out the potential benefits of adjusting feeding rates in order to compensate for a low protein ration. Endogenous differences in growth rate associated with segregated phenotypes were mainly accounted for by differences in energy acquisition, with feeding rates differing by ~ 2-fold between fast and slow growers. Additionally, significant differences were recorded for the unitary metabolic costs (i.e., per unit of metabolizable energy), indicating that higher metabolic efficiency was also a component of faster growth.

Key words: growth phenotypes, protein/energy ratio, *Ruditapes philippinarum*, scope for growth.

Introduction

Selective breeding is one fundamental step in aquaculture practices oriented to the generation of stocks exhibiting improved traits for animal production. For commercial species of marine bivalve mollusks, faster growth has been considered of utmost interest since the variability in growth rate of bivalves ranks among the highest in the animal kingdom (Goff, 2011), and much of this variation has been reported to be genetically determined

(Dégremont et al., 2005; Evans and Langdon, 2006; Toro and Paredes, 1996). In the context of a joint research project (FIGEBIV, MINECO 2013) centered around one important mariculture species (the Manila clam *Ruditapes philippinarum*), we have undertaken the analysis of this endogenous component of growth by combining physiological and genetic approaches for a) the identification of physiological components of growth variability and b) the search for candidate genes accounting for differential growth phenotypes. The desire for an experimental system appropriate to assess genotype-phenotype associations for growth traits in the context of this project has encouraged the creation of families combined with the selection of intrafamily growth groups.

Methods based on the quantification of physiological parameters liable to be subsequently integrated in an energy budget (the SFG approach) have proven to be useful in the identification of feeding and metabolic behavior traits that are mainly responsible for inherent differences in growth performance among groups of individuals conforming to differentiated growth categories of possible genetic origin (Bayne, 2000; Bayne et al., 1999b, 1999a; Fernández-Reiriz et al., 2016; Tamayo et al., 2016, 2014, 2011). As systematized by Bayne (1999), such persistent physiological differences have been reported to comprise variable capacities for both energy acquisition (feeding and digestive behavior) and energy savings associated with metabolic processes of maintenance and growth. The existence of such a strong genetic component, however, does not exclude phenotypic plasticity in the form of a flexible physiological response to ambient fluctuations, particularly food availability as the main environmental determinant of rates of growth (Bayne, 2004). Consequently, a thorough analysis of adaption capabilities to the food environment exhibited by selected groups of bivalves would require assessment of a) the extent to which physiological behavior

underlying growth performance is genetically determined and b) how much of this behavior can, on its own, be environmentally modulated in order to achieve the more effective exploitation of available food resources within the limits set by the genetic constitution of individuals (Prieto et al., 2018; Tamayo et al., 2015).

In addressing the interactions between food supply and the growth rate of bivalves, several distinctive features of the food environment should be considered, especially those concerning the quantity and quality of available seston (Gosling, 2015). The main source of food in suspension feeding bivalves is assumed to be phytoplankton, and the growth of both natural and cultivated populations generally exhibits a good correlation with phytoplankton abundance, as represented by Chl *a* concentration in the water column (Figueiras et al., 2002; Pieters et al., 1980; Smaal and Van Stralen, 1990). However, different studies performed over the last few decades have emphasized the importance of other components of the seston, including mainly organic detritus together with bacteria and zooplankton (Arapov et al., 2010; Huang et al., 2003; Langdon and Newell, 1990). Concerning this point, trophic analysis of natural populations of bivalves (see Hawkins et al., 2013 for a review) has revealed that the amount of energy available in the seston (=POM) required to achieve a given growth performance increases as a function of the relative abundance of organic detritus in the diet, very likely reflecting the poor nutritional value of these materials relative to phytoplankton as a consequence of differences in biochemical composition and specifically the higher C:N ratio of detritus.

Bivalve growth is not only dependent on food density (Rico-Villa et al., 2009) but also on the balance between different constituents (e.g., Brown et al., 1998; Wikfors et al., 1992) and, in fact, gross biochemical composition indices have been extensively used in order to assess physiological condition (Lucas and Beninger, 1985). Among the major biochemical

constituents (i.e., proteins, carbohydrates and lipids) in diets, proteins are more directly related to growth due to their metabolic and structural functions and may consequently become a limiting factor, as suggested by both laboratory (Brown et al., 1997; Hawkins and Bayne, 1991; Ibarrola et al., 1996; Romberger and Epifanio, 1981) and field (Bayne, 2009; Gremare et al., 1997) studies. Hence, a direct connection between protein input and growth should be appreciated (Kreeger and Langdon, 1993).

As evidence of potential N limitation in bivalves, the cockle *Cerastoderma edule* absorbs nitrogen more efficiently than overall organic matter (Urrutia et al., 1996) and absorbs proteins better than lipids (Ibarrola et al., 2000), pointing to a compensatory mechanism for strict N requirements. Similarly, protein utilization relative to energy, as measured in terms of the respective net growth efficiencies, tends to increase under conditions of food limitation (negative energy balance) in the mussel *Mytilus edulis*, suggesting the conservation of protein deposition rates at the expense of energy (Hawkins and Bayne, 1991), while higher conversion efficiencies for protein appear on the basis of faster growth in selected oysters (*Saccostrea commercialis*) relative to controls (Bayne, 2000).

Experiments with doubly labeled (^{15}N and ^{14}C) protein in the diet (Kreeger et al., 1996, 1995) provided evidence of a noticeable feature concerning the metabolic fate of dietary protein in mussels (*Mytilus edulis*), offering a metabolically based mechanism for some of the above observations: the higher assimilation efficiency (90% of the absorbed ration) of the N isotope (the amino-N fraction) compared to less than 34% of the C isotope (the amino-C fraction) (Kreeger et al., 1996) suggests the intensive use of ingested proteins to fuel the N pool through transamination reactions for protein synthesis, with the consequent waste of most of the amino-C fraction, possibly as a component of metabolic fecal loss

(Hawkins and Bayne, 1985). In energy terms, this poses a heavy tax on the use of dietary protein for protein deposition into the tissues, to add to the elevated metabolic costs of protein synthesis (Lee et al., 2016; Pan et al., 2018).

Given these high energy requirements involved in dietary protein utilization for the growth of bivalve tissues, two issues are relevant in the context of the present study:

- 1) How does changing food quality (C:N ratio), expressed as the protein to energy ratio in the diet, impact growth rate, estimated by means of the energy balance (the SFG), and which physiological components of growth are involved in that response?
- 2) How do physiological adjustments to diet quality become modulated in order to fulfill the contrasting energy requirements set by the occurrence of intrinsic differences in growth performance (i.e., fast vs. slow growing phenotypes)?

To address these questions, four differentiated growth phenotypes of Manila clam juveniles, obtained through combined interfamilial and intrafamilial segregation, were conditioned to two diets differing broadly in terms of their protein to energy ratios. Then, the physiological components of the energy balance, and resulting SFG, were determined and compared between these growth groups during both the acute and chronic responses to changing biochemical composition of the food.

2. - Materials and methods

2.1 Families and growth groups

Manila clam specimens used in this study belonged to the offspring of two families (1 and 8) from a set of full-sib families established for the combined characterization of growth rate, physiological parameters and SNP polymorphisms, in order to identify QTLs

related to growth and growth-associated physiological components of the energy balance. Groups of sibs (families from now on) were obtained from pair matings, which were performed in May 2015 at the IRTA hatchery. Larvae from each mating were cultured in 300 L tanks at 21°C, and fed *Isochrysis galbana* at 10000 cells mL⁻¹. Water was changed every 48h. Larvae from six matings survived until settlement. After completion of metamorphosis, spat from each family was transferred to 5 L containers with mesh bottom, which were suspended in 500 L tanks with running seawater, first at the IRTA facilities, and after they reached 3 mm, at the IATS facilities. When they reached a minimum size of 7 mm (December 2015), 85 clams were sampled randomly from each family, they were labeled, and their shell length and height were measured. Labeled animals were redistributed in five 50 L tanks provided with substrate (fine-grained sand) and kept at a density of 340 individuals per square meter until the final sampling (June 2017), while fed a diet of *Tetraselmis suecica* supplemented with *Isochrysis galbana* and *Chaetoceros* sp. Two families were chosen for this study on the basis of their growth rate (see below).

The preliminary characterization of growth performance of these families (in terms of regression of growth rate vs. body size) indicated a 47.6% higher growth rate in Family 1 relative to Family 8. For the specific objectives of this study, two growth groups were segregated inside each family by choosing the larger and smaller specimens to which the conditions of fast (F) and slow (S) growth, respectively, were assigned. Table 1 shows the sizes and characteristics of these groups determined in order to fulfill the requirements of the experimental design: some 30 individuals per growth group presenting the highest degree of size-homogeneity possible (CV ranged from 7% in F to 16% in S groups). Size differences

achieved between F and S groups were similar for both families:, i.e., ~ 2x in terms of shell length and 6x in terms of live weight.

Table 1. Mean (SD) size of the four groups of clams before starting the experiments

family	Growth group	N	Length (mm)	Weight (mg)
1	F	30	23.14 (1.54)	2359.05 (468.63)
1	S	27	12.36 (1.93)	380.63 (138.36)
8	F	30	21.92 (1.91)	2095.39 (602.52)
8	S	34	11.4 (1.96)	314.07 (150.41)

2.2 Maintenance and experimental design

After arrival at the laboratory of Animal Physiology (UPV/EHU, 21st June 2017), these groups were separately maintained for 10 days in a 50 L tank filled with aerated seawater (34 PPT) regulated at 17 °C and fed *Isochrysis galbana* (T-ISO clone) at a cell concentration equivalent to 1 mm³ L⁻¹ (~20,000 cells mL⁻¹). Water in the tank was changed daily.

In these experiments, we tested the responses of clams from different families and growth groups to diets that differed in biochemical composition and that were based on cultures of the microalgal species *Rhodomonas lens* growing in the exponential phase (Diets N+) or maintained in the stationary phase of the culture (Diets N-). A basic outline of the experimental design is presented in Figure 1: each of the aforementioned F and S groups was homogenously divided ($F=0.21$, $p=0.893$) into four subgroups for subsequent diet treatments, and each clam was numbered for individual determinations of growth and physiological parameters. Each of these groups was food-conditioned (acclimated) to the

179 diets N+ or N- for 15 days (Table 2). Subsequently, each member of the pair conditioned to
180 diets N+ or N- was exposed to one of the experimental diets based on exponential (E) or
181 stationary (S) cultures for physiological determination, resulting in 4 experimental conditions
182 for each growth group and family (Figure 1 and Table 3). In the notation of these categories,
183 the first letter indicates the diet used in acclimation, and the second letter indicates the
184 experimental diets used for the acute exposure prior (3 d) and during physiological
185 determination. That is, each group*family combination was analyzed under the four
186 nutritional scenarios stated in *Acute exposure* in Figure 1: N+N+, N+N-, N-N+ and N-N;
187 using different pools of clams under each condition (i.e., no repeated measurements were
188 carried out).

Figure 1.- Experimental setup for the recording of physiological parameters in the fast (F) and slow (S) growing groups segregated from two families. Five individuals (n=5) were used in each of the 16 resulting groups.

2.3 Composition of diets

The basic component of diets was the microalgae *R. lens* in either exponential or stationary phase. E microalgae were obtained in a continuous culture system, in which 20% of the stock was renewed daily. To obtain S microalgae, cultures that had reached the

exponential phase were maintained in a static culture system without further addition of nutrients until the stationary phase was reached. The turning point for the transition from the E to S stage of culture was identified by the color change of the culture (from red to green), indicative of N limitation.

Table 2. Diet composition, including the culture phase of R. lens, C:N index and protein/energy ratio

Diet	Culture phase	C:N	Protein/Energy (P/E, $\mu\text{g J}^{-1}$)
N+	E (Exponential)	4.94 (0.21)	24.77 (0.53)
N-	S (Stationary)	14.54 (0.22)	8.11 (0.12)

Tables 2 and 3 show the composition and characteristics of two types of diets used in this study: the acclimation diets used in food conditioning of clams prior to experimentation and the experimental diets used in the acute exposure of clams during physiological measurements. The composition of the acclimation diets included only microalgae in either the E or S phase of culture. The composition of the experimental diets was based also on these microalgae as food but included 35-40% inorganic content (by weight) to fulfill the requirements of an inorganic tracer in absorption efficiency (AE) determinations by the Conover method. The inorganic component consisted of silt particles $<63 \mu\text{m}$ obtained from surficial sediment samples collected in the field that were ashed at 450°C for organic matter combustion. Hence, experimental diets were prepared by mixing both microalgae and silt particles in the stated proportions with the aid of a magnetic stirrer and then dosed with a peristaltic pump. Both acclimation and experimental diets were dosed at approx. 1 and $1.25 \text{ mm}^3 \text{ L}^{-1}$, respectively, in terms of particles packed volume, to achieve a POM concentration of 0.6 mg L^{-1} under each condition.

Elemental analysis of diets was conducted during the acclimation period, as well as in the course of experiments, on samples collected over preweighted glass fiber filters (GF/C) by filtering a known volume of water from the feeding tanks and washing with 50 mL of seawater. Samples were immediately frozen at -20°C, lyophilized, and maintained at -20°C until they were analyzed in an Euro EA Elemental Analyzer (CHNS) from EuroVector, using acetanilide as a standard. The protein/energy ratio was indirectly estimated as follows: protein content of the sample (μg) was estimated by using the equivalence $P=N * 5.8$ (Gnaiger and Bitterlich, 1984), while energy content (J) was estimated as the product of POM and the energy equivalents (22.906 and 26.826 J mg^{-1} for E and S cultures, respectively; Platt and Irwin, 1973).

Table 3. Characteristics of both acclimation and exposure diets, where TPM is total particulate matter, PIM is particulate inorganic matter, POM is particulate organic matter, OC is organic content ($=\text{POM}/\text{TPM}$) and C:N is the carbon to nitrogen index.

	Diet	TPM (mg L ⁻¹)	PIM (mg L ⁻¹)	POM (mg L ⁻¹)	OC	C:N
Acclimation diets	N+	0.64 (0.15)	0.09 (0.07)	0.55 (0.08)	0.87 (0.07)	4.94 (0.21)
	N-	0.56 (0.05)	0.05 (0.01)	0.51 (0.04)	0.92 (0.02)	14.54 (0.22)
Diet composition in the acute exposure of the different experimental conditions	N+N+	1.13 (0.25)	0.53 (0.19)	0.60 (0.09)	0.54 (0.08)	5.40
	N+N-	1.25 (0.24)	0.48 (0.09)	0.77 (0.16)	0.62 (0.03)	10.70
	N-N+	1.20 (0.25)	0.56 (0.19)	0.64 (0.09)	0.54 (0.08)	5.43
	N-N-	1.00 (0.15)	0.35 (0.08)	0.65 (0.11)	0.65 (0.07)	13.57

Characterization of food suspensions leading to the data in Table 3 was carried out twice per week in triplicate during the acclimation period and 5-6 times in triplicate during the exposure. For this purpose, samples of water collected from the feeding tanks were filtered through preweighted glass fiber filters (GF/C), rinsed with ammonium formate (0.9% w/v) to prevent salt retention and dried for 24-48 h at 100°C to estimate dry weight. Ash

weight was computed after calcination for 6 h at 450°C. Total particulate matter (TPM, mg L⁻¹) and particulate inorganic matter (PIM, mg L⁻¹) were calculated from the dry weight and ash weight of material retained in the filters, respectively, and the difference TPM – PIM represented the particulate organic matter (POM, mg L⁻¹).

2.4 Physiological determinations

Physiological determinations were performed individually, with five individual samples for each condition. Measurements involved in the quantification of components of the energy balance lasted 4 days for each experimental condition.

The clearance rate (CR, L h⁻¹) was measured by the flow-through chamber method (Crisp, 1971), where clams were individually placed in a 125 mL flask with a constant supply of diet. Flow rates through the flasks were regulated to produce reductions in particle concentrations in the range of 15-30%, corresponding to conditions for which CR is independent of the flow rate (Filgueira et al., 2006). Twelve to 16 such measurements were registered during the daytime (from 8 a.m. to 8 p.m.) by means of a particle counter (Beckman Z1 Counter), and the CR of each individual was estimated as the average of these measurements. The organic ingestion rate (OIR, mg h⁻¹) was then computed as the product of CR and POM.

Absorption efficiency (AE, decimal units) was estimated by the method of (Conover, 1966) from the organic content of food suspensions and the feces produced in the course of CR measurements. Both water samples and feces were filtered on GF/C filters and processed for total dry weight and inorganic weight determinations as described previously (section

259 2.3). Organic content (OC) was computed as organic weight (= total - inorganic) divided by
260 total weight.

261 The absorption rate (AR, mg h^{-1}) of organic matter was estimated as the product of
262 OIR and AE, and the energy equivalents that were applied to the absorbed ration in SFG
263 computation were those described in Section 2.3.

264 The metabolic rate was assessed as the oxygen consumption rate (VO_2 , $\mu\text{L O}_2 \text{ h}^{-1}$).
265 Clams were individually placed in 150 mL chambers filled with filtered seawater at a
266 constant temperature (17°C) sealed with LDO oxygen probes connected to a Hatch HQ40d
267 oximeter. Rates of oxygen consumption were computed from the decline in oxygen
268 concentration in the chambers registered over 3-4 h. A chamber without animals was used as
269 a control. These rates were converted to energy equivalents (J h^{-1}) by using the following
270 oxi-caloric coefficient: $1 \text{ mL O}_2 = 20.08 \text{ J}$ (Gnaiger, 1983).

271 For determination of ammonia excretion rates ($\text{VNH}_4\text{-N}$, $\mu\text{g NH}_4\text{-N h}^{-1}$), animals
272 were located individually in open flasks with 30 mL of filtered seawater ($0.2 \mu\text{m}$ Millipore
273 membranes) for 2-3 h, and the ammonia concentration was determined according to the
274 phenol-hypochlorite method (Solórzano, 1969). Two flasks without animals were used as
275 controls. Rates of ammonia excretion were converted to energy equivalents (U: J h^{-1}) by
276 using a conversion factor of 24.853 J mg^{-1} (Elliott and Davison, 1975).

277 The O:N index was calculated as the proportion between atomic equivalents of
278 oxygen consumed and nitrogen excreted by each animal.

279 The scope for growth (SFG, J h^{-1}) was estimated as the following difference: $\text{AR} -$
280 $(\text{R} + \text{U})$

After physiological determinations were concluded, clams were dissected, gill area was estimated by image analysis with Fiji software (Schindelin et al., 2012), and soft tissues were lyophilized to obtain dry weight measurements. Growth in terms of energy was indirectly estimated by the conversion factor of 23.96 J mg⁻¹ (Álvarez-Jorna, 1995). For comparative purposes, physiological rates were standardized to a common tissue dry weight of 85.95 mg (the average value), using scaling factors (*b*) obtained in a previous experiment of 0.609, 0.697 and 1.00 to scale CR, VO₂ and VNH₄-N, respectively, to soft body weight (own unpublished data). Likewise, a mass exponent of 2.00 was used to standardize gill area to a common length.

Statistical analysis

This study comprises the analysis of the effects of 4 factors on the suite of physiological traits involved in growth rate, including a) Two endogenous factors associated with differences in growth performance between families (family factor) and with the effects of size segregation (growth category factor). b) Two exogenous factors corresponding to differences in the biochemical composition of the acclimation diet prior to physiological experiments (acclimation diet factor) or the actual diet ingested during physiological determinations (exposure diet factor). Physiological measurements recorded under this experimental design were compared for significant differences through a 4-way ANOVA using R (R Core Team, 2016), after the data were tested for normality (Shapiro-Wilk) and homoscedasticity (Levene). Relationships between different components of energy balance as well as between SFG and actual growth rates were fitted through linear regression analyses (by least squares) using the same software.

3. - Results:

3.1 Comparison of means of pooled values corresponding to factors under analysis

Means of pooled values of the physiological components of the energy balance, gill areas and O:N indices, computed for alternative values of the 4 factors (categories) referred to above, are presented in Table 4. Table 5 summarizes the results of the corresponding 4-way ANOVA, including terms for both single factors and factor interactions up to the 4th degree.

Table 4.- Means of pooled values (SE) of different parameters computed for alternative values of the factors under study: family (F;1 vs. 8); growth group (G;F vs. S); acclimation diet (A;N+ vs. N-) and exposure diet (E;N+ vs. N-). CR=clearance rate ($L h^{-1}$); GA=gill area (mm^2); AE=absorption efficiency (decimal units); AR=absorption rate ($J h^{-1}$); R=metabolic rate ($J h^{-1}$); U=nitrogen excretion rate ($J h^{-1}$); SFG=slope for growth ($J h^{-1}$); O:N=oxygen:nitrogen index (atomic ratio).

		CR	AE	AR	R	U	O:N	SFG	GA
A		0.83	0.72	9.91	1.36	0.17	21.49	8.38	406.26
	N+	(0.05)	(0.02)	(0.57)	(0.08)	(0.02)	(3.28)	(0.58)	(7.14)
		0.8	0.65	8.06	1.3	0.17	41.28	6.6	361.52
E	N-	(0.05)	(0.02)	(0.45)	(0.11)	(0.03)	(6.87)	(0.44)	(10.2)
		0.89	0.81	10.28	1.48	0.27		8.52	
	N+	(0.05)	(0.01)	(0.57)	(0.09)	(0.03)	12 (1.39)	(0.59)	-
G		0.75	0.56	7.69	1.17	0.06	50.77	6.46	
	N-	(0.05)	(0.01)	(0.39)	(0.09)	(0.01)	(6.46)	(0.41)	-
		0.95	0.67	10.3	1.44	0.12	36.35	8.74	370.57
F	F	(0.05)	(0.03)	(0.52)	(0.09)	(0.02)	(5.26)	(0.52)	(7.68)
		0.68	0.7	7.67	1.21	0.22	26.42	6.24	397.22
	S	(0.04)	(0.02)	(0.46)	(0.09)	(0.03)	(5.84)	(0.47)	(10.61)
F		0.91	0.67	9.72	1.3	0.17	27.16	8.24	369.58
	1	(0.04)	(0.02)	(0.51)	(0.11)	(0.03)	(4.73)	(0.52)	(9.38)
		0.73	0.7	8.25	1.35	0.16	35.61	6.74	398.2
F	8	(0.05)	(0.02)	(0.53)	(0.08)	(0.03)	(6.3)	(0.52)	(9.07)

CR and gill area: These two parameters are considered together on account of the functional relationship linking the filtering activity with the surface area of the filtering organ. Both endogenous factors (family and growth category) are associated with significant differences in CR and gill area, although trends are not strictly concordant: for example, offspring of Family 1 exhibit approximately 20% higher CR compared with that of Family 8, and F clams present a similar difference with respect to S clams (irrespective of family ascription). Conversely, gill areas tend to be higher in S clams and Family 8, and these differences are clearly less sharp relative to CR differences but are still significant. On the other hand, the very significant positive influence of acclimation to diets N+ on the gill area (Table 5 and Figure 2b) partly supports the effect that clams tend to feed faster with this diet, especially following a period of acclimation (Table 4).

Absorption efficiency and absorption rate: Each of the factors under study exerted significant differences ($p < 0.001$) on absorption efficiency. However, even if significant, effects of endogenous factors (family and growth group) *per se* appear quantitatively irrelevant compared with the strong effect of actual dietary condition, resulting, for instance, in a 44% increase observed in the absorption efficiency of clams exposed to diets N+ relative to diets N- (Table 4). The complex behavior of this parameter in the acute vs. chronic response to changing diet composition in each group of clams results in a set of combined effects (interactions; Table 5) that will be described in the next section. Absorption rate behavior combines the effects of feeding rates (CR) and absorption efficiencies. Consequently, AR values exhibited substantial significant differences (Table 5) for each factor (acclimation, exposure, growth group and family). Compared with diets N-, feeding diets N+ promoted an increase in the AR, both in the acute response (33% increase) and

during acclimation (23%) (Table 4). Concerning the endogenous factors, differences in feeding rates caused greater AR values in F relative S clams or in clams from Family 1 compared with those of Family 8 (Table 4).

Metabolic expenditures (R and U) and O:N index: Both metabolic and N excretion rates increase significantly with acute exposure to N+ diets (Table 4 and 5), but this effect is considerably higher for U (350%) than for R (26%). Consequently, values of the O:N index experienced a 4-fold decline in clams fed this high N diet. Overall, acclimation to N+ diets also promoted a significant reduction in the O:N index (Table 5), although this effect was only noticeable during acute exposure to N- diets (Figure 4c). This behavior is accounted for by the consistent significance of the exposure*acclimation interaction term for R, U and the O:N index (Table 5).

Endogenous factors (family and growth group) had no significant effects on metabolic rate or the O:N index, although F clams registered, on average, 19% more metabolic activity than S clams. Rates of ammonia excretion were significantly higher (~2-fold) in S than F clams, but no differences between Family 1 and 8 were recorded.

SFG: SFG integrates a diversity of effects on physiological components and was significantly affected for all tested variables, both endogenous and exogenous (dietary). Confirming their status as fast growers, F clams had significantly higher SFG than S clams, while those belonging to Family 1 had higher SFG than clams from Family 8. On the other hand, both acute and chronic exposure to N-rich diets promoted a significant increase in the SFG, with acclimation enhancing the effects of the acute change (see acclimation*exposure interaction term in Table 5).

3.2 Combined dynamics of the acute and chronic responses

Figures 2a to 5a have been designed to represent the dynamics of the different physiological parameters combining the acute and chronic (acclimation) responses to changes in the N content of the diet. Each point (with standard deviation bars) represents the mean (n=5) value of each group, in which different clams were used, whereas lines connecting these points for the N+N+, N+N-, N-N-, N-N+ and N+N+ sequence of experimental conditions are drawn to model the acute-chronic response to dietary change.

Figure 2a) Size-standardized values of clearance rate in fast (black) and slow (gray) growing clams belonging to families 1 (solid lines) and 8 (dotted lines); b) size-standardized gill area values of fast (dark) and slow (light) growing clams from both families and acclimated to N+ and N- diets.

The main dietary effects on CR are accounted for by the acclimation*exposure interaction (Table 5), conforming to a general pattern in which the acute change of the diet

(either from N+ to N- and *vice versa*) results in a decline in feeding rate followed by a recovery along the acclimation period (“W shaped pattern”; Figure 2a). In addition, acute exposure to the low-nitrogen diet had a higher impact on CR than did the change from N- to N+, leading to bigger decreases in the feeding activity. The magnitude of these changes tends to be greater in Family 1 than Family 8, with maximal differences between the S groups of both families.

Figure 3a) Absorption efficiency and b) absorption rate values of fast (black) and slow (gray) growing clams belonging to families 1 (solid lines) and 8 (dotted lines)

AE shows a positive dependence on the N content of the diet, with a general U-shaped pattern in which the acute response (i.e., a strong reduction in AE following the change from N+ to N- diets) is reinforced during the acclimation period (Figure 3a). However, these effects are smaller in slow growing clams (S), which are able to maintain their AE values relatively stable along the acclimation phase, resulting in higher efficiencies of S clams with the low-N diet.

Rates of absorption (AR) approximately follow this same “U-shaped” trend (Figure 3b), with some deviations from the general pattern due to the differential behavior of CR between families and growth groups: while F clams exposed to N+ diets rapidly recovered from reduced AR values achieved during chronic exposure to N-poor diets, the response of S clams required much longer acclimation periods.

Figure 4 Size-standardized values of a) metabolic rate (VO_2) and b) ammonia excretion rate of fast (black) and slow (gray) growing clams belonging to families 1 (solid lines) and 8 (dotted lines); c) O:N index of each group (Acclim: acclimation diet; Exp: exposure diet)

Rates of energy expenditure (both metabolic and excretion rates) showed a rather common pattern of response to combined acute and chronic changes in N content of the diet (Figure 4). In general, acute change involving improved nutritional conditions (from N- to N+) results in a positive effect on these rates, leading to maximal values that are maintained or reduced (depending on growth group or family) during the acclimation. Following the acute decline in the N+ to N- change, acclimation to the N- diet resulted in an additional minor reduction in excretion rates, these changes being greater for S than for F clams.

The combined effects of acute exposure and acclimation to diets with different N contents on SFG fit different patterns for F and S clams (Figure 5; see also acclimation*exposure*growth group interaction in Table 5). For F clams, acute decline following the N+ to N- change is further reinforced during acclimation to the poor diet, while the increasing response to the opposite acute change is maintained along the acclimation to the N+ diet. This U-shaped pattern would indicate that the SFG trend of F clams is governed by AE behavior. For S clams, any change in diet quality (either from N- to N+ or *vice versa*) resulted in a decline of SFG values in the acute response, followed by a recovery during the acclimation phase. This W-shaped pattern would indicate that the SFG trend in slow growing clams is governed by CR behavior.

Figure 5 Size-standardized SFG values in fast (black) and slow (gray) growing clams belonging to families 1 (solid lines) and 8 (dotted lines).

3.3 The relationship between SFG and actual growth rate (GR)

The potential of SFG methodology to predict actual growth rates (GR) was tested by performing regression analysis of both measurements (Figure 6). For this purpose, only physiological measurements recorded under fully acclimated conditions were employed, assuming that weight changes used in actual growth measurements would reflect the stable conditions achieved in acclimated specimens. The fitted regression equation was $SFG = 1.03 GR + 2.19$ ($F=52.9$, $p<0.001$), in which the slope did not significantly differ from 1 ($F=0.0366$ $p=0.8494$), but intercept was significantly different from 0 ($F=4.2396$ $p=0.04639$), reflecting a slight overestimation of SFG over actual growth. Nevertheless, the weak significance ($p=0.046$) concerning the deviation of the intercept from 0 is indicative of a good concordance between both measurements and would confirm the validity of SFG methodology in predicting the growth rate.

Figure 6 Relationship between SFG and actual growth for fast (black) and slow (gray) growing clams. The solid line represents $y=x$, while the dashed line is a plot of the regression equation fitted to the experimental data.

441 *Table 5. Summary of 4-way ANOVA testing of the significant effects of acclimation and exposure to alternative diets, growth category*
 442 *and family on gill area and physiological parameters. Significant differences ($p < 0.05$) are highlighted in bold characters.*

	CR	Gill area	AE	AR	R	VNH ₄ -N	SFG	O:N
Acclimation	$F = 0.368, p = 0.546$	$F = 14.949, p < 0.001$	$F = 106.456, p < 0.001$	$F = 14.243, p < 0.001$	$F = 0.202, p = 0.655$	$F = 0, p = 0.989$	$F = 11.451, p = 0.001$	$F = 13.579, p < 0.001$
Exposure	$F = 8.228, p = 0.006$	-	$F = 1199.494, p < 0.001$	$F = 57.89, p < 0.001$	$F = 6.594, p = 0.013$	$F = 88.567, p < 0.001$	$F = 15.122, p < 0.001$	$F = 52.109, p < 0.001$
Growth category	$F = 31.335, p < 0.001$	$F = 5.305, p = 0.025$	$F = 14.949, p < 0.001$	$F = 25.778, p < 0.001$	$F = 3.548, p = 0.064$	$F = 18.561, p < 0.001$	$F = 22.495, p < 0.001$	$F = 3.414, p = 0.069$
Family	$F = 13.032, p = 0.001$	$F = 6.12, p = 0.016$	$F = 15.454, p < 0.001$	$F = 8.536, p = 0.005$	$F = 0.148, p = 0.701$	$F = 0.259, p = 0.613$	$F = 8.135, p = 0.006$	$F = 2.476, p = 0.121$
Acclimation:Exposure	$F = 29.94, p < 0.001$	-	$F = 0.502, p = 0.481$	$F = 13.532, p < 0.001$	$F = 6.936, p = 0.011$	$F = 9.952, p = 0.002$	$F = 16.999, p < 0.001$	$F = 20.479, p < 0.001$
Acclimation:Growth category	$F = 0.886, p = 0.35$	$F = 0.441, p = 0.509$	$F = 12.999, p = 0.001$	$F = 3.688, p = 0.059$	$F = 0.52, p = 0.474$	$F = 1.009, p = 0.319$	$F = 5.347, p = 0.024$	$F = 2.229, p = 0.14$
Exposure:Growth category	$F = 0.146, p = 0.703$	-	$F = 26.049, p < 0.001$	$F = 1.399, p = 0.241$	$F = 2.025, p = 0.16$	$F = 15.061, p < 0.001$	$F = 1.631, p = 0.206$	$F = 0.001, p = 0.971$
Acclimation:family	$F = 0.29, p = 0.592$	$F = 1.666, p = 0.201$	$F = 0.929, p = 0.339$	$F = 0.005, p = 0.942$	$F = 1.015, p = 0.317$	$F = 3.087, p = 0.084$	$F = 0.083, p = 0.774$	$F = 2.813, p = 0.098$
Exposure:family	$F = 0.167, p = 0.684$	-	$F = 19.741, p < 0.001$	$F = 3.028, p = 0.087$	$F = 1.226, p = 0.272$	$F = 0.117, p = 0.734$	$F = 1.544, p = 0.219$	$F = 1.876, p = 0.176$
Type of seed:family	$F = 0.012, p = 0.915$	$F = 0.925, p = 0.34$	$F = 9.759, p = 0.003$	$F = 0.091, p = 0.764$	$F = 0.088, p = 0.767$	$F = 1.554, p = 0.217$	$F = 0.253, p = 0.617$	$F = 0.071, p = 0.79$
Acclimation:Exposure:Growth category	$F = 0.911, p = 0.344$	-	$F = 30.004, p < 0.001$	$F = 5.319, p = 0.024$	$F = 0.367, p = 0.547$	$F = 0.021, p = 0.885$	$F = 5.356, p = 0.024$	$F = 0.638, p = 0.427$
Acclimation:Exposure:family	$F = 2.928, p = 0.092$	-	$F = 4.146, p = 0.046$	$F = 1.324, p = 0.254$	$F = 2.563, p = 0.114$	$F = 2.685, p = 0.106$	$F = 2.506, p = 0.118$	$F = 4.524, p = 0.037$
Acclimation:Growth category:family	$F = 0.071, p = 0.79$	$F = 0.233, p = 0.631$	$F = 13.829, p < 0.001$	$F = 0.84, p = 0.363$	$F = 0.219, p = 0.641$	$F = 0.91, p = 0.344$	$F = 0.506, p = 0.479$	$F = 0.409, p = 0.525$
Exposure: Growth category:family	$F = 1.112, p = 0.296$	-	$F = 0.003, p = 0.958$	$F = 1.005, p = 0.32$	$F = 3.009, p = 0.088$	$F = 0.697, p = 0.407$	$F = 1.925, p = 0.17$	$F = 0.288, p = 0.594$
Acclimation:Exposure: Growth category:family	$F = 2.166, p = 0.146$	-	$F = 0.355, p = 0.553$	$F = 3.129, p = 0.082$	$F = 0.93, p = 0.338$	$F = 2.105, p = 0.152$	$F = 2.372, p = 0.128$	$F = 1.421, p = 0.238$

4. - Discussion:

The aims of this study were mainly to test the effect of diet quality, given as the nitrogen to energy ratio (or inversely, the C:N index), on features of the physiological behavior underlying variability among differentiated growth phenotypes. To experimentally address this question concerning the dependence of growth rate of bivalves on dietary food value, several procedures have been attempted to obtain a range of biochemical profiles. These include the use of different microalgal species (Albentosa et al., 1996; Enright et al., 1986; Epifanio, 1979; Fernández-Reiriz et al., 2015; Pettersen et al., 2010; Walne, 1970), mixtures of microalgae with inert organic particles (Albentosa et al., 2002, 1999; Maeda-Martínez et al., 2016; Pérez-Camacho et al., 1998) or manufactured microcapsules (Kreeger et al., 1996, 1995; Kreeger and Langdon, 1993) as well as the manipulation of phytoplankton cultures for the specific purpose of changing the protein content of the cell (Kreeger and Langdon, 1994, 1993; Uriarte and Fariás, 1999; Utting, 1985). This last procedure has the advantage of relying mainly on differences in biochemical composition, while other differential features related to the physical constitution of particles that might affect the rates of food processing would be virtually absent. In this study, two different diets were made up from the same species of phytoplankton (*Rhodomonas lens*) cultivated either in the exponential or stationary phase to achieve a 2.5-fold difference in the protein content (C:N ratios of 4.9 and 12.8 in diets N⁺ and N⁻, respectively). The transition from the exponential (N⁺) to the stationary (N⁻) phase of the culture was observed to result in an increase in cell size (from 44.2 to 82.5 pg cell⁻¹), but food supply in our experiments was not regulated to the same cell number but rather to achieve the same organic ration (mg POM L⁻¹) in both diets, and gill retention efficiency has been reported to be constant (near 100%) in that size range (Defossez and Hawkins, 1997; Ward and Shumway, 2004); hence, we generated the hypothesis that

differences in physiological behavior observed between both diets respond solely to differences in their biochemical composition.

Early observations concerning limitations exerted by N availability on energy flows within coastal environments (Mann, 1982), as well as the positive relationship between protein ingestion and production exhibited by marine invertebrates (Roman, 1983), offer an appropriate reference context for the present finding that acclimation to N⁺ diets promoted a higher growth rate than acclimation to N⁻ diets in juveniles of the Manila clam. This confirms previous results concerning the positive correlation reported between dietary protein content of experimental diets and growth rate in the early life stages of many different species of bivalves (Brown et al., 1998; Enright et al., 1986; Kreeger and Langdon, 1993; Maeda-Martínez et al., 2016; Uriarte and Fariás, 1999; Utting, 1986; Wikfors et al., 1992), including juveniles of the Manila clam (*Ruditapes philippinarum*) (Albentosa et al., 2002; Langton et al., 1977) and the con-generic *R. decussatus* (Albentosa et al., 1999). In the specific case of Manila clams, Gallager and Mann (1981) reported a negative impact on growth for diets presenting C:N ratios above 10.5. Thus, actively growing bivalves appear to require moderate to high levels of dietary protein to optimize growth, whereas diet quality (the protein to energy P/E ratio) has been reported to be a better predictor of growth performance than the overall food ration (Kreeger and Langdon, 1993).

4.1 Acute vs. chronic response to changing dietary N content

In the present experiments, groups of clams were conditioned for 15 days to N⁺ or N⁻ diets, and then physiological parameters and the resulting SFG were recorded for each acclimation group with both N⁺ and N⁻ diets. The obtained set of data could thus be arranged to generate a sequence comprising the acute followed by the chronic response

of physiological parameters to every change from N+ to N- and *vice versa* (see Figures 2a to 5a). Growth rate differences found between N+ and N- diets were accounted for by differences in physiological behavior regarding the main components of the energy balance and features of this behavior, including both short and long-term responses to dietary change. Characteristically, the acute-chronic sequence varies for the different physiological parameters, depicting a complex pattern of food conditioning. For instance, net energy gain (the absorption rate: AR) was governed by the contrasting behavior of the feeding rate and absorption efficiency: feeding rates declined with every change in the diet (either N+ to N- or *vice versa*), and full achievement required acclimation, whereas AE increased in the acute change to the N+ diet and further improved during the acclimation to that diet. Patterns of metabolic energy expenditure were characterized by the increase in both oxygen consumption and ammonia excretion in the acute change from N- to N+, which partly declines during the acclimation to the N-rich diet.

Values of physiological parameters recorded under corresponding acclimation diets (i.e., the N+N+ and N-N- experimental sets) would be representative of stable conditions after diet acclimation, and computed SFG from these values can consequently be assumed to indicate growth performance exhibited by the different groups. Comparison of physiological behavior of clams fully conditioned to N+ and N- diets, across the different family * growth group combinations (Figure 7), indicate significantly higher rates of both energy gain and loss and resulting SFG values that were increased by 50% on average for clams fed the high-protein diet, with the only exception being the S8 (slow growers of Family 8) group.

Figure 7: Bar plot reporting components of net energy gain (sum of all categories) and loss (R: gray bars, U: white bars) and resulting SFG (dark gray bars) in the different family * growth group combinations fully acclimated to diets N+ and N-.

While the beneficial effect of increased protein/energy (P/E) indices of the diet on the growth rate of bivalves has been broadly documented (see references above), there is presently a noticeable lack of experimental evidence in this group concerning the concomitant effects on the energy budget and the physiological components of growth that are involved in the improvement of individual production. In this respect, commercial fish species might provide a useful reference for comparative purposes since the energetic response to variable E/P diets has been frequently tested (Bendiksen et al., 2002; Boujard

and Médale, 1994; Helland and Grisdale-Helland, 1998; Morales et al., 1994): For instance, data from experiments performed on the rainbow trout fed high and low P/E diets designed on an iso-energetic basis (Saravanan et al., 2012) agree with the present results regarding the positive effects of protein-rich diets on feed intake (= OIR), digestible energy intake (=AR) and energy retention (=SFG), with nonsignificant differences in metabolic heat output associated with diet. The same results obtained in such different aquacultured animal models are indicative of common mechanisms and point to limitations of the homeostatic control of protein income, exemplified for instance by the fact that specimens exposed to low P/E diets do not resort to “overeating” to compensate for reduced dietary protein, with a resulting reduction in growth performance.

Figure 8 Absorption efficiency (AE) as a function of organic ingestion (OIR). Lines were fitted to mean values for the different groups of clams fully acclimated to N+ (closed circles) and N- (open circles) diets.

Further analysis of the physiological components of energy gain in the present experiments suggests that the above limitations might stem from digestive constraints. Increased energy income with high-quality (high P/E) food relies partly on a behavioral response since the feeding rate has increased significantly by the term of acclimation to N+ relative to N- diets (by 12% on average). However, the outstanding effect of protein-rich diets on energy balance is mediated by a strong increase in the AE, which rises by nearly 80% (from 0.51 to 0.85) during the change from fully acclimated N- to N+. Although the AE of N (AE_N) has been reported to be higher than that of C (AE_C) under several circumstances (Bayne, 2009; Urrutia et al., 1996), consideration of this factor could not fully account for differences in AE for overall organics of the magnitude found in this study, since N absorption contributes at most 20% to the total absorption of organics. Consequently, broad differences in digestive performance (*sensu* Navarro et al., 2009) on both types of food particles should be invoked to account for a more efficient absorption of *R. lens* cells in the exponential (E) relative to the stationary (S) phase of the culture. Since most of the change (~80%) has already occurred in the short-term response (see Figure 3a), variable digestibility must rely on differential features of both microalgal cells (e.g., biochemical constitution or, eventually, size), rather than be based on enzyme induction processes that might take place during acclimation. This interpretation is consistent with the different behaviors exhibited by E and S cells upon digestion (Figure 8): while the AE of E microalgae appears to be virtually independent of the ingestion rate, that of S microalgae declines with rising ingestion, revealing that digestive yield is strongly dependent on the gut residence time of food particles. This feature of the N- diet, a characteristic of poorly digestible food, would have the effect of canceling out the benefits of any potential increase in the feeding rate oriented to compensate for the low protein ration.

Feeding on different P/E diets has a neat effect on rates of energy expenditure (both oxygen consumption and ammonia excretion rates), although the energetic relevance of these dietary effects is lower, with the SFG response mainly driven by energy gain processes (as it will be discussed later). Two main points would summarize the present results concerning energy expenditure: 1) Acclimation to a high-protein diet increases both metabolic and N excretion rates, with the stronger change being achieved in the acute response. 2) These dietary effects are much higher for rates of excretion relative to the metabolic response, resulting in a maximum decrease of the O:N ratio by a factor of 7.5 when clams acclimated to the N- diet are fed the N+ diet. The corresponding difference in O:N ratios between clams fully acclimated to N+ and N- was a factor of 5.1.

Determination of ammonia excretion in studies regarding the scope for growth determination has been traditionally neglected in bivalves since its representation in the energy budget is considered low (1-10% of total metabolic energy expenditure in *M. edulis*; Bayne and Newell, 1983). However, this measurement gains interest in the context of studies—such as the present study—testing the effect of variable protein/energy inputs on the components of the energy balance, given that ammonia excretion represents a summary output of dietary protein metabolism. The reason, provided by studies reported in the Introduction section (see Kreeger et al., 1996, 1995), is that the preferred pathway for protein assimilation in bivalves appears to comprise incorporation to the N pool through transamination reactions, rather than the most direct incorporation to the pool of essential amino acids for protein synthesis. Consequently, Langton et al., (1977) reported in *Tapes japonica* (= *Ruditapes philippinarum*) a 2-fold increase in ammonia excretion corresponding to a 3.5-fold increase in N-protein ingestion, similar to the present results with the same species, where a 2.7-fold increase in N ingestion led to an 8.7-times

increase in N excretion. A positive dependence of rates of ammonia excretion on dietary protein ingestion generally has been documented in other aquatic animals, such as fishes (Brunty et al., 1997; Green and Hardy, 2008; Porter et al., 1987), pointing to a certain identity concerning the mechanisms of protein assimilation in ammoniotelic organisms. In line with the present approach, a 2 to 3-fold increase in the rate of N excretion has been reported in response to increasing dietary P/E ratios by the same factor in both shrimps (Coelho et al., 2019; Gauquelin et al., 2007) and fishes (Saravanan et al., 2012).

Consideration of the extent to which the stoichiometric C:N coupling between the diet and growing tissues occurs might provide further understanding of the observed diet-dependent behavior of N excretion. Bayne (2017) put forward a stoichiometric hypothesis fitting experimental data for *Crassostrea gigas* (Bayne, 2009; Mao et al., 2006): “When feeding behaviour cannot fully compensate for an imbalance between C:N of the tissues and C:N of the diet, and nitrogen is absorbed in excess of the demand, then this excess is removed by excretion. Similarly, if insufficient N is absorbed then nitrogen excretion is reduced in order to conserve tissue nitrogen”. In the present case, N surplus resulting from the elevated energy inputs achieved to sustain high growth demands with the N+ diet would account for rates of ammonia excretion that exceed 10 times the rates recorded with the N- diet, in which low protein content combines with reduced AE to doubly constrain N absorption.

4.2 Endogenous factors

Separate breeding of two families differing in growth rate and size-segregation inside each family was combined in this study to achieve a wide range of growth phenotypes for physiological determinations and SFG computation. In general,

physiological differences accounting for growth variation were found to be higher for the size-segregation factor than the family factor: For instance, based on means of pooled values (Table 4), net energy gain values differed by 34% between fast (F) and slow (S) clams, while the corresponding difference between F1 and F8 amounted only to 18%; the equivalent figures for the SFG were 40 and 22%, respectively. These trends occur irrespective of the diet, since interaction terms have null or weak significance (Table 5). Energy losses, when significantly different, showed the opposite behavior. Thus, the energy balances for the different family * growth group combinations rank as follows: $F.1 > F.8 > S.1 > S.8$

The relative contribution of components of energy gain and loss to SFG variation is illustrated in Figure 9. Clearly, energy balance fluctuations recorded across diets and growth phenotypes are overwhelmingly driven by the physiological processes of feeding and absorption, while the effects of metabolic energy expenditure are virtually null (regression equations for either respiration or ammonia excretion rates were only significant on their intercepts). Previous studies analyzing SFG fluctuations across size-segregated growth groups of clams (*R. philippinarum*: Tamayo et al., 2011) and mussels (*M. galloprovincialis*: Fernández-Reiriz et al., 2016) also reported that fast growth was mainly accounted (80- 90%) for by increased energy gain, while 10-20% was explained by changes in metabolism. This agrees with the general observation that limits to growth in bivalves are set primarily by functional constraints on feeding and digestion rather than by the associated metabolic costs (Bayne et al., 1989; Navarro et al., 1992), although metabolic constraints have been reported at low food concentrations (Albentosa et al., 1996; Beiras et al., 1994), i.e., when food rations approach the maintenance conditions.

Figure 9 Net energy gain (AR) and loss (R and U) at different levels of the SFG. Lines fitted (minimum squares) to individual data for all experimental sets in this study.

Physiological parameters accounting for SFG fluctuations were the same, irrespective of diet acclimation, and involved both rates of energy acquisition and conversion efficiencies:

1) Increased energy acquisition in faster growers was fully accounted for by the higher feeding rates found in F clams (40% increase with respect to S clams) and clams from Family 1 (25% increase with respect to those of Family 8), since the AE was found to decline (little but significantly; Table 5) in fast growers relative to slow growers. Several studies comparing the feeding behavior of size-segregated growth groups have reported that faster feeding of F specimens correlated with larger gills in both clams (Tamayo et al., 2011) and mussels (Prieto et al., 2018). The present results preclude any generalization of this kind of relationship as gill areas were found in this case to be consistently higher in the groups of clams exhibiting lower clearance rates (i.e., in fam. 8

654 compared with fam. 1 and in S clams compared with F clams), suggesting that a greater
 655 pumping capacity per unit of surface area (or increased gill efficiency) would be an
 656 alternative mechanism to achieve fast feeding. On the other hand, gill area was found to
 657 differentiate during diet acclimation (higher values corresponded to the protein-rich diet),
 658 an adaptive response similar to gill and palp size adjustments to different food
 659 environments revealed in transplant experiments of different species of bivalves
 660 (Tedengren et al., 1990; Worrall and Widdows, 1983). This points to a highly plastic trait
 661 (Honkoop et al., 2003) and does not support the idea, implicit in previous studies (Prieto
 662 et al., 2019, 2018; Tamayo et al., 2011), that gill size would be a constitutive trait, liable
 663 *per se* to account for interindividual differences in feeding and growth rates. The ability
 664 to adapt the size of filtering structures was noticeably greater in F clams and clams of
 665 Family 1 (see Figure 2b), and this differential behavior might explain the prompt and
 666 more efficient feeding adjustments exhibited by fast growers during the dietary changes.
 667 Generally, F/Fam.1 clams lost less feeding and absorption capacity with diet N- and
 668 recovered earlier their previous level of activity with diet N+ than did the S/Fam.8 clams.
 669 This, combined with more restrained energy losses, resulted in fast growers achieving a
 670 better management of energy resources during nutritional fluctuations.

671 2) Lack of significant differences in rates of metabolic energy expenditure
 672 recorded for the different growth phenotypes implies that increased energy gain (2 to 3-
 673 fold increase in rates of absorption between fast and slow growers) does not occur at the
 674 expense of greater metabolic outputs, thus pointing to variable metabolic efficiency
 675 (Bayne, 2004, 1999). Indeed, the unitary metabolic costs (i.e., per unit of metabolizable
 676 energy or AR) were found to decline for rising SFG (Figure 10), indicating that greater
 677 metabolic efficiencies also stood out as a component of faster growth. Statistical
 678 comparison (ANOVA) of mean values for these unitary costs between the different family

* growth group combinations indicates significant differences between families, where Family 1 sibs attained 77% lower unitary costs than Family 8 sibs ($F=6.486$, $p=0.0159$). Similar results concerning interfamily differences in metabolic efficiency have also been reported in the mussel *Perna canaliculus* (Ibarrola et al., 2017).

Figure 10.- Unitary metabolic costs (computed individually) as a function of SFG (open circles), and mean values of this relationship in the different growth phenotypes (squares: F clams, triangles: S clams; black symbols: Family 1, gray symbols: Family 8), under fully acclimated conditions. Superscripts indicate significant differences ($p < 0.05$) in terms of unitary costs

Therefore, the present results confirmed most earlier studies on bivalves reporting selection for faster growth to entail faster rates of feeding and absorption (increased energy acquisition), most frequently coupled to increased metabolic efficiency represented by the reduced metabolic costs per unit of absorption (Bayne, 2000, 1999; Bayne et al., 1999b, 1999a; Fernández-Reiriz et al., 2016; Holley and Foltz, 1987;

Ibarrola et al., 2017; Pernet et al., 2008; Tamayo et al., 2016, 2015, 2014, 2011; Toro and Vergara, 1998). Bayne (2000) and Pace et al. (2006) have convincingly associated these variations in costs of growth with differences in the efficiency of protein deposition in both larvae and adult oysters. In spite of very different experimental approaches used in the segregation of growth phenotypes, a noticeable uniformity regarding the complex of physiological processes underlying differential growth appears to be the rule across those studies. Moreover, this endogenous component of growth variability has been found to subsume a wide range of phenotypic plasticity for physiological traits, expressed in the form of the present feeding and digestive adjustments to a change in the biochemical composition of food, as well as equivalent responses reported in variable nutritional (Bayne, 2000; Tamayo et al., 2015) or thermal (Tamayo et al., 2013) contexts.

Aknowledgements:

This work was supported by the Spanish Ministry of Economy and Competitiveness (AGL2013-49144-C3-1-R). K. Arranz was funded by a predoctoral research grant from Universidad del País Vasco/Euskal Herriko Unibertsitatea (UPV/EHU). SGiker technical and human support (UPV/EHU, MICINN, GV/EJ, ESF) is gratefully acknowledged.

References:

- Albentosa, M., Fernández-Reiriz, M.J., Pérez-Camacho, A., Labarta, U., 1999. Growth performance and biochemical composition of *Ruditapes decussatus* (L.) spat fed on microalgal and wheatgerm flour diets. J. Exp. Mar. Biol. Ecol. 232, 23–37. [https://doi.org/10.1016/S0022-0981\(98\)00086-0](https://doi.org/10.1016/S0022-0981(98)00086-0)
- Albentosa, M., Pérez-Camacho, A., Fernández-Reiriz, M.J., Labarta, U., 2002. Wheatgerm flour in diets for Manila clam, *Ruditapes philippinarum*, spat. Aquaculture 212, 335–345. [https://doi.org/10.1016/S0044-8486\(02\)00121-7](https://doi.org/10.1016/S0044-8486(02)00121-7)

- 719 Albentosa, M., Pérez-Camacho, A., Labarta, U., Fernández-Reiriz, M.J., 1996.
- 720 Evaluation of live microalgal diets for the seed culture of *Ruditapes decussatus*
- 721 using physiological and biochemical parameters. *Aquaculture* 148, 11–23.
- 722 [https://doi.org/10.1016/S0044-8486\(96\)01405-6](https://doi.org/10.1016/S0044-8486(96)01405-6)
- 723 Álvarez-Jorna, P., 1995. Crecimiento, reproducción y energética fisiológica de la almeja
- 724 *Tapes philippinarum* (PhD Thesis). Ph. D. thesis (unpublished). University of the
- 725 Basque Country (UPV/EHU), Bilbao, Spain.
- 726 Arapov, J., Ezgeta-Balić, D., Peharda, M., Ninčević Gladan, Ž., 2010. Bivalve feeding—
- 727 how and what they eat? *Ribarstvo* 68, 105–116.
- 728 Bayne, B., 2017. *Biology of Oysters*, 1st ed. *Developments in Aquaculture and Fisheries*
- 729 Science vol. 41, Academic Press, Elsevier.
- 730 Bayne, B.L., 2009. Carbon and nitrogen relationships in the feeding and growth of the
- 731 Pacific oyster, *Crassostrea gigas* (Thunberg). *J. Exp. Mar. Biol. Ecol.* 374, 19–
- 732 30.
- 733 Bayne, B.L., 2004. Phenotypic flexibility and physiological tradeoffs in the feeding and
- 734 growth of marine bivalve molluscs. *Integr. Comp. Biol.* 44, 425–432.
- 735 Bayne, B.L., 2000. Relations between variable rates of growth, metabolic costs and
- 736 growth efficiencies in individual Sydney rock oysters (*Saccostrea commercialis*).
- 737 *J. Exp. Mar. Biol. Ecol.* 251, 185–203. [https://doi.org/10.1016/S0022-](https://doi.org/10.1016/S0022-0981(00)00211-2)
- 738 [0981\(00\)00211-2](https://doi.org/10.1016/S0022-0981(00)00211-2)
- 739 Bayne, B.L., 1999. Physiological Components of Growth Differences between Individual
- 740 Oysters (*Crassostrea gigas*) and a Comparison with *Saccostrea commercialis*.
- 741 *Physiol. Biochem. Zool.* 72, 705–713. <https://doi.org/10.1086/316714>

- 742 Bayne, B.L., Hawkins, A.J.S., Navarro, E., Iglesias, I.P., 1989. Effects of seston
743 concentration on feeding, digestion and growth in the mussel *Mytilus edulis*. Mar.
744 Ecol. Prog. Ser. 47–54.
- 745 Bayne, B.L., Hedgecock, D., McGoldrick, D., Rees, R., 1999a. Feeding behaviour and
746 metabolic efficiency contribute to growth heterosis in Pacific oysters [*Crassostrea*
747 *gigas* (Thunberg)]. J. Exp. Mar. Biol. Ecol. 233, 115–130.
748 [https://doi.org/10.1016/S0022-0981\(98\)00125-7](https://doi.org/10.1016/S0022-0981(98)00125-7)
- 749 Bayne, B.L., Newell, R.C., 1983. Physiological Energetics of Marine Molluscs, in:
750 Saleuddin, A.S.M., Wilbur, K.M. (Eds.), The Mollusca. Academic Press, pp. 407–
751 515. <https://doi.org/10.1016/B978-0-12-751404-8.50017-7>
- 752 Bayne, B.L., Svensson, S., Nell, J.A., 1999b. The Physiological Basis for Faster Growth
753 in the Sydney Rock Oyster, *Saccostrea commercialis*. Biol. Bull. 197, 377–387.
754 <https://doi.org/10.2307/1542792>
- 755 Beiras, R., Camacho, A.P., Albentosa, M., 1994. Comparison of the scope for growth
756 with the growth performance of *Ostrea edulis* seed reared at different food
757 concentrations in an open-flow system. Mar. Biol. 119, 227–233.
- 758 Bendiksen, E., Jobling, M., Arnesen, A.M., 2002. Feed intake of Atlantic salmon parr
759 *Salmo salar* L. in relation to temperature and feed composition. Aquac. Res. 33,
760 525–532.
- 761 Boujard, T., Médale, F., 1994. Regulation of voluntary feed intake in juvenile rainbow
762 trout fed by hand or by self-feeders with diets containing two different
763 protein/energy ratios. Aquat. Living Resour. 7, 211–215.
- 764 Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties
765 of microalgae for mariculture. Aquaculture 151, 315–331.

- 766 Brown, M.R., McCausland, M.A., Kowalski, K., 1998. The nutritional value of four
767 Australian microalgal strains fed to Pacific oyster *Crassostrea gigas* spat.
768 Aquaculture 165, 281–293. [https://doi.org/10.1016/S0044-8486\(98\)00256-7](https://doi.org/10.1016/S0044-8486(98)00256-7)
- 769 Brunty, J.L., Bucklin, R.A., Davis, J., Baird, C.D., Nordstedt, R.A., 1997. The influence
770 of feed protein intake on tilapia ammonia production. Aquac. Eng. 16, 161–166.
- 771 Coelho, R.T.I., Yasumaru, F.A., Passos, M.J.A.C.R., Gomes, V., Lemos, D., 2019.
772 Energy budgets for juvenile Pacific whiteleg shrimp *Litopenaeus vannamei* fed
773 different diets. Braz. J. Oceanogr. 67.
- 774 Conover, R.J., 1966. Assimilation of organic matter by zooplankton. Limnol Ocean. 11,
775 338–345.
- 776 Crisp, D.J., 1971. Energy flow measurements., in: Methods for the Study of Marine
777 Benthos. (Holme, N. A. and Mc Inture, A. D. Eds.). Blackwell, Oxford, pp. 197–
778 323.
- 779 Defossez, J.-M., Hawkins, A.J.S., 1997. Selective feeding in shellfish: size-dependent
780 rejection of large particles within pseudofaeces from *Mytilus edulis*, *Ruditapes*
781 *philippinarum* and *Tapes decussatus*. Mar. Biol. 129, 139–147.
- 782 Dégremont, L., Bédier, E., Soletchnik, P., Ropert, M., Huvet, A., Moal, J., Samain, J.-F.,
783 Boudry, P., 2005. Relative importance of family, site, and field placement timing
784 on survival, growth, and yield of hatchery-produced Pacific oyster spat
785 (*Crassostrea gigas*). Aquaculture 249, 213–229.
786 <https://doi.org/10.1016/j.aquaculture.2005.03.046>
- 787 Elliott, J.M., Davison, W., 1975. Energy equivalents of oxygen consumption in animal
788 energetics. Oecologia 19, 195–201.

- 789 Enright, C.T., Newkirk, G.F., Craigie, J.S., Castell, J.D., 1986. Evaluation of
790 phytoplankton as diets for juvenile *Ostrea edulis* L. J. Exp. Mar. Biol. Ecol. 96,
791 1–13.
- 792 Epifanio, C.E., 1979. Growth in bivalve molluscs: Nutritional effects of two or more
793 species of algae in diets fed to the American oyster *Crassostrea virginica*
794 (Gmelin) and the hard clam *Mercenaria mercenaria* (L.). Aquaculture 18, 1–12.
795 [https://doi.org/10.1016/0044-8486\(79\)90095-4](https://doi.org/10.1016/0044-8486(79)90095-4)
- 796 Evans, S., Langdon, C., 2006. Effects of genotype x environment interactions on the
797 selection of broadly adapted Pacific oysters (*Crassostrea gigas*). Aquaculture
798 261, 522–534.
- 799 Fernández-Reiriz, M.J., Irisarri, J., Labarta, U., 2016. Flexibility of physiological traits
800 underlying inter-individual growth differences in intertidal and subtidal mussels
801 *Mytilus galloprovincialis*. PloS One 11, e0148245.
- 802 Fernández-Reiriz, M.J., Irisarri, J., Labarta, U., 2015. Feeding behaviour and differential
803 absorption of nutrients in mussel *Mytilus galloprovincialis*: Responses to three
804 microalgae diets. Aquaculture 446, 42–47.
805 <https://doi.org/10.1016/j.aquaculture.2015.04.025>
- 806 Figueiras, F.G., Labarta, U., Reiriz, M.F., 2002. Coastal upwelling, primary production
807 and mussel growth in the Rías Baixas of Galicia, in: Sustainable Increase of
808 Marine Harvesting: Fundamental Mechanisms and New Concepts. Springer, pp.
809 121–131.
- 810 Filgueira, R., Labarta, U., Fernandez-Reiriz, M.J., 2006. Flow-through chamber method
811 for clearance rate measurements in bivalves: design and validation of individual
812 chambers and mesocosm. Limnol. Oceanogr. Methods 4, 284–292.

- 813 Gallagher, S.M., Mann, R., 1981. The effect of varying carbon/nitrogen ratio in the
814 phytoplankter *Thalassiosira pseudonana* (3H) on its food value to the bivalve
815 *Tapes japonica*. Aquaculture 26, 95–105. [https://doi.org/10.1016/0044-](https://doi.org/10.1016/0044-8486(81)90113-7)
816 8486(81)90113-7
- 817 Gauquelin, F., Cuzon, G., Gaxiola, G., Rosas, C., Arena, L., Bureau, D.P., Cochard, J.-
818 C., 2007. Effect of dietary protein level on growth and energy utilization by
819 *Litopenaeus stylirostris* under laboratory conditions. Aquaculture 271, 439–448.
- 820 Gnaiger, E., 1983. Heat dissipation and energetic efficiency in animal anoxibiosis:
821 economy contra power. J. Exp. Zool. Part Ecol. Genet. Physiol. 228, 471–490.
- 822 Gnaiger, E., Bitterlich, G., 1984. Proximate biochemical composition and caloric content
823 calculated from elemental CHN analysis: a stoichiometric concept. Oecologia 62,
824 289–298.
- 825 Goff, S.A., 2011. A unifying theory for general multigenic heterosis: energy efficiency,
826 protein metabolism, and implications for molecular breeding. New Phytol. 189,
827 923–937.
- 828 Gosling, E., 2015. Marine bivalve molluscs, 2nd ed. John Wiley & Sons.
- 829 Green, J.A., Hardy, R.W., 2008. The effects of dietary protein: energy ratio and amino
830 acid pattern on nitrogen utilization and excretion of rainbow trout *Oncorhynchus*
831 *mykiss* (Walbaum). J. Fish Biol. 73, 663–682.
- 832 Gremare, A., Amouroux, J.M., Charles, F., Dinet, A., Riaux-Gobin, C., Baudart, J.,
833 Medernach, L., Bodiou, J.Y., Vetion, G., Colomines, J.C., 1997. Temporal
834 changes in the biochemical composition and nutritional value of the particulate
835 organic matter available to surface deposit-feeders: a two year study. Mar. Ecol.
836 Prog. Ser. 150, 195–206.

- 837 Hawkins, A.J.S., Bayne, B.L., 1991. Nutrition of marine mussels: factors influencing the
838 relative utilizations of protein and energy. *Aquaculture, The Biology and*
839 *Cultivation of Mussels* 94, 177–196. [https://doi.org/10.1016/0044-](https://doi.org/10.1016/0044-8486(91)90117-P)
840 8486(91)90117-P
- 841 Hawkins, A.J.S., Bayne, B.L., 1985. Seasonal variation in the relative utilization of
842 carbon and nitrogen by the mussel *Mytilus edulis*: budgets, conversion efficiencies
843 and maintenance requirements. *Mar. Ecol. Prog. Ser.* Oldendorf 25, 181–188.
- 844 Hawkins, A.J.S., Pascoe, P.L., Parry, H., Brinsley, M., Cacciatore, F., Black, K.D., Fang,
845 J.G., Jiao, H., Mcgonigle, C., Moore, H., 2013. Comparative feeding on
846 chlorophyll-rich versus remaining organic matter in bivalve shellfish. *J. Shellfish*
847 *Res.* 32, 883–898.
- 848 Helland, S.J., Grisdale-Helland, B., 1998. The influence of replacing fish meal in the diet
849 with fish oil on growth, feed utilization and body composition of Atlantic salmon
850 (*Salmo salar*) during the smoltification period. *Aquaculture* 162, 1–10.
- 851 Holley, M.E., Foltz, D.W., 1987. Effect of multiple-locus heterozygosity and salinity on
852 clearance rate in a brackish-water clam, *Rangia cuneata* (Sowerby). *J. Exp. Mar.*
853 *Biol. Ecol.* 111, 121–131.
- 854 Honkoop, P.J.C., Bayne, B.L., Drent, J., 2003. Flexibility of size of gills and palps in the
855 Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) and the Pacific oyster
856 *Crassostrea gigas* (Thunberg, 1793). *J. Exp. Mar. Biol. Ecol.* 282, 113–133.
- 857 Huang, S.-C., Kreeger, D.A., Newell, R.I.E., 2003. Tidal and seasonal variations in the
858 quantity and composition of seston in a North American, mid-Atlantic saltmarsh.
859 *Estuar. Coast. Shelf Sci.* 56, 547–560.

- 860 Ibarrola, I., Hilton, Z., Ragg, N.L., 2017. Physiological basis of inter-population, inter-
861 familiar and intra-familiar differences in growth rate in the green-lipped mussel
862 *Perna canaliculus*. *Aquaculture* 479, 544–555.
- 863 Ibarrola, I., Iglesias, J., Navarro, E., 1996. Differential absorption of biochemical
864 components in the diet of the cockle *Cerastoderma edule*: enzymatic responses to
865 variations in seston composition. *Can. J. Zool.* 74, 1887–1897.
- 866 Ibarrola, I., Navarro, E., Urrutia, M.B., 2000. Acute and acclimated digestive responses
867 of the cockle *Cerastoderma edule* (L.) to changes in food quality and quantity: I.
868 Feeding and absorption of biochemical components. *J. Exp. Mar. Biol. Ecol.* 252,
869 181–198. [https://doi.org/10.1016/S0022-0981\(00\)00233-1](https://doi.org/10.1016/S0022-0981(00)00233-1)
- 870 Kreeger, D.A., Hawkins, A.J.S., Bayne, B.L., 1996. Use of dual-labeled microcapsules to
871 discern the physiological fates of assimilated carbohydrate, protein carbon, and
872 protein nitrogen in suspension-feeding organisms. *Limnol. Oceanogr.* 41, 208–
873 215.
- 874 Kreeger, D.A., Hawkins, A.J.S., Bayne, B.L., Lowe, D.M., 1995. Seasonal variation in
875 the relative utilization of dietary protein for energy and biosynthesis by the mussel
876 *Mytilus edulis*. *Mar. Ecol. Prog. Ser.* 177–184.
- 877 Kreeger, D.A., Langdon, C.J., 1994. Digestion and assimilation of protein by *Mytilus*
878 *trossulus* (Bivalvia: Mollusca) fed mixed carbohydrate/protein microcapsules.
879 *Mar. Biol.* 118, 479–488.
- 880 Kreeger, D.A., Langdon, C.J., 1993. Effect of dietary protein content on growth of
881 juvenile mussels, *Mytilus trossulus* (Gould 1850). *Biol. Bull.* 185, 123–139.
- 882 Langdon, C.J., Newell, R.I., 1990. Utilization of detritus and bacteria as food sources by
883 two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel
884 *Geukensia demissa*. *Mar. Ecol. Prog. Ser.* 299–310.

- 885 Langton, R.W., Winter, J.E., Roels, O.A., 1977. The effect of ration size on the growth
886 and growth efficiency of the bivalve mollusc *Tapes japonica*. Aquaculture 12,
887 283–292.
- 888 Lee, J.W., Applebaum, S.L., Manahan, D.T., 2016. Metabolic cost of protein synthesis in
889 larvae of the Pacific oyster (*Crassostrea gigas*) is fixed across genotype,
890 phenotype, and environmental temperature. Biol. Bull. 230, 175–187.
- 891 Lucas, A., Beninger, P.G., 1985. The use of physiological condition indices in marine
892 bivalve aquaculture. Aquaculture 44, 187–200.
- 893 Maeda-Martínez, A.N., Saucedo, P.E., Mazón-Suástegui, J.M., Acosta-Salmón, H.,
894 Romero-Meléndez, Z., 2016. Scope for growth of juvenile Cortez oyster,
895 *Crassostrea corteziensis* fed isocaloric diets made up with microalgae and cereal
896 flours. Submiss. Artic. Platf.-Lat. Am. J. Aquat. Res. 44.
- 897 Mann, K.H., 1982. Ecology of Coastal Waters: A Systems Approach, California Library
898 Reprint Series. University of California Press. 322 pp.
- 899 Mao, Y., Zhou, Y., Yang, H., Wang, R., 2006. Seasonal variation in metabolism of
900 cultured Pacific oyster, *Crassostrea gigas*, in Sanggou Bay, China. Aquaculture
901 253, 322–333.
- 902 Morales, A.E., Cardenete, G., De la Higuera, M., Sanz, A., 1994. Effects of dietary
903 protein source on growth, feed conversion and energy utilization in rainbow trout,
904 *Oncorhynchus mykiss*. Aquaculture 124, 117–126.
- 905 Navarro, E., Iglesias, J.I.P., Ortega, M.M., 1992. Natural sediment as a food source for
906 the cockle *Cerastoderma edule* (L.): effect of variable particle concentration on
907 feeding, digestion and the scope for growth. J. Exp. Mar. Biol. Ecol. 156, 69–87.

- 908 Navarro, E., Méndez, S., Ibarrola, I., Urrutia, M.B., 2009. Comparative utilization of
909 phytoplankton and vascular plant detritus by the cockle *Cerastoderma edule*:
910 digestive responses during diet acclimation. *Aquat. Biol.* 6, 247–262.
- 911 Pace, D.A., Marsh, A.G., Leong, P.K., Green, A.J., Hedgecock, D., Manahan, D.T., 2006.
912 Physiological bases of genetically determined variation in growth of marine
913 invertebrate larvae: A study of growth heterosis in the bivalve *Crassostrea gigas*.
914 *J. Exp. Mar. Biol. Ecol.* 335, 188–209.
915 <https://doi.org/10.1016/j.jembe.2006.03.005>
- 916 Pan, T.-C.F., Applebaum, S.L., Frieder, C.A., Manahan, D.T., 2018. Biochemical bases
917 of growth variation during development: a study of protein turnover in pedigreed
918 families of bivalve larvae (*Crassostrea gigas*). *J. Exp. Biol.* 221, jeb171967.
- 919 Pérez-Camacho, A., Albentosa, M., Fernández-Reiriz, M.J., Labarta, U., 1998. Effect of
920 microalgal and inert (cornmeal and cornstarch) diets on growth performance and
921 biochemical composition of *Ruditapes decussatus* seed. *Aquaculture* 160, 89–
922 102.
- 923 Pernet, F., Tremblay, R., Redjah, I., Sévigny, J.-M., Gionet, C., 2008. Physiological and
924 biochemical traits correlate with differences in growth rate and temperature
925 adaptation among groups of the eastern oyster *Crassostrea virginica*. *J. Exp. Biol.*
926 211, 969–977.
- 927 Pettersen, A.K., Turchini, G.M., Jahangard, S., Ingram, B.A., Sherman, C.D., 2010.
928 Effects of different dietary microalgae on survival, growth, settlement and fatty
929 acid composition of blue mussel (*Mytilus galloprovincialis*) larvae. *Aquaculture*
930 309, 115–124.

- 931 Pieters, H., Kluytmans, J.H., Zandee, D.I., Cadee, G.C., 1980. Tissue composition and
932 reproduction of *Mytilus edulis* in relation to food availability. Neth. J. Sea Res.
933 14, 349–361.
- 934 Platt, T., Irwin, B., 1973. Caloric content of phytoplankton. Limnol. Oceanogr. 18, 306–
935 310.
- 936 Porter, C.B., Krom, M.D., Robbins, M.G., Brickell, L., Davidson, A., 1987. Ammonia
937 excretion and total N budget for gilthead seabream (*Sparus aurata*) and its effect
938 on water quality conditions. Aquaculture 66, 287–297.
- 939 Prieto, D., Markaide, P., Urrutxurtu, I., Navarro, E., Artigaud, S., Fleury, E., Ibarrola, I.,
940 Urrutia, M.B., 2019. Gill transcriptomic analysis in fast-and slow-growing
941 individuals of *Mytilus galloprovincialis*. Aquaculture 734242.
- 942 Prieto, D., Urrutxurtu, I., Navarro, E., Urrutia, M.B., Ibarrola, I., 2018. *Mytilus*
943 *galloprovincialis* fast growing phenotypes under different restrictive feeding
944 conditions: Fast feeders and energy savers. Mar. Environ. Res. 140, 114–125.
945 <https://doi.org/10.1016/j.marenvres.2018.05.007>
- 946 R Core Team, 2016. R: A Language and Environment for Statistical Computing. R
947 Foundation for Statistical Computing, Viena-, Austria.
- 948 Rico-Villa, B., Pouvreau, S., Robert, R., 2009. Influence of food density and temperature
949 on ingestion, growth and settlement of Pacific oyster larvae, *Crassostrea gigas*.
950 Aquaculture 287, 395–401. <https://doi.org/10.1016/j.aquaculture.2008.10.054>
- 951 Roman, M.R., 1983. Nitrogenous nutrition of marine invertebrates, in: Carpenter, E.J.,
952 Capone, D.G. (Eds.), Nitrogen in the Marine Environment. Academic Press,
953 London, pp. 347–383.

- 954 Romberger, H.P., Epifanio, C.E., 1981. Comparative effects of diets consisting of one or
955 two algal species upon assimilation efficiencies and growth of juvenile oysters,
956 *Crassostrea virginica* (Gmelin). Aquaculture 25, 77–87.
- 957 Saravanan, S., Schrama, J.W., Figueiredo-Silva, A.C., Kaushik, S.J., Verreth, J.A.,
958 Geurden, I., 2012. Constraints on energy intake in fish: the link between diet
959 composition, energy metabolism, and energy intake in rainbow trout. Plos One 7,
960 e34743.
- 961 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T.,
962 Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., 2012. Fiji: an open-source
963 platform for biological-image analysis. Nat. Methods 9, 676–682.
- 964 Smaal, A.C., Van Stralen, M.R., 1990. Average annual growth and condition of mussels
965 as a function of food source, in: North Sea—Estuaries Interactions. Springer, pp.
966 179–188.
- 967 Solórzano, L., 1969. Determination of ammonia in natural waters by the
968 phenolhypochlorite method. Limnol. Oceanogr. 14, 799–801.
- 969 Tamayo, D., Azpeitia, K., Markaide, P., Navarro, E., Ibarrola, I., 2016. Food regime
970 modulates physiological processes underlying size differentiation in juvenile
971 intertidal mussels *Mytilus galloprovincialis*. Mar. Biol. 163, 131.
- 972 Tamayo, D., Ibarrola, I., Cigarría, J., Navarro, E., 2015. The effect of food conditioning
973 on feeding and growth responses to variable rations in fast and slow growing spat
974 of the Manila clam (*Ruditapes philippinarum*). J. Exp. Mar. Biol. Ecol. 471, 92–
975 103.
- 976 Tamayo, D., Ibarrola, I., Navarro, E., 2013. Thermal dependence of clearance and
977 metabolic rates in slow-and fast-growing spats of manila clam *Ruditapes*
978 *philippinarum*. J. Comp. Physiol. B 183, 893–904.

- 979 Tamayo, D., Ibarrola, I., Urrutia, M.B., Navarro, E., 2011. The physiological basis for
980 inter-individual growth variability in the spat of clams (*Ruditapes philippinarum*).
981 Aquaculture 321, 113–120.
- 982 Tamayo, D., Ibarrola, I., Urrutxurtu, I., Navarro, E., 2014. Physiological basis of extreme
983 growth rate differences in the spat of oyster (*Crassostrea gigas*). Mar. Biol. 161,
984 1627–1637.
- 985 Tedengren, M., André, C., Johannesson, K., Kautsky, N., 1990. Genotypic and
986 phenotypic differences between Baltic and North Sea populations of *Mytilus*
987 *edulis* evaluated through reciprocal transplantations. III. Physiology. Mar. Ecol.
988 Prog. Ser. 221–227.
- 989 Toro, J.E., Paredes, L.I., 1996. Heritability estimates of larval shell length in the Chilean
990 blue mussel *Mytilus chilensis*, under different food densities. Aquat. Living
991 Resour. 9, 347–350.
- 992 Toro, J.E., Vergara, A.M., 1998. Growth and Heterozygosity in a 12-Month-Old Cohort
993 of *Ostrea chilensis* Obtained by Mass Spawning in the Laboratory. Mar. Ecol. 19,
994 311–323.
- 995 Uriarte, I., Fariás, A., 1999. The effect of dietary protein content on growth and
996 biochemical composition of Chilean scallop *Argopecten purpuratus* (L.)
997 postlarvae and spat. Aquaculture 180, 119–127.
- 998 Urrutia, M.B., Iglesias, J.I.P., Navarro, E., Prou, J., 1996. Feeding and absorption in
999 *Cerastoderma edule* under environmental conditions in the bay of
1000 Marennesoleron (Western France). J. Mar. Biol. Assoc. U. K. 76, 431–450.
- 1001 Utting, S.D., 1986. A preliminary study on growth of *Crassostrea gigas* larvae and spat
1002 in relation to dietary protein. Aquaculture 56, 123–138.

- 2918
2919
2920 1003 Utting, S.D., 1985. Influence of nitrogen availability on the biochemical composition of
2921
2922 1004 three unicellular marine algae of commercial importance. Aquac. Eng. 4, 175–
2923
2924 1005 190.
2925
2926 1006 Walne, P.R., 1970. Studies on the food value of nineteen genera of algae to juvenile
2927
2928 1007 bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria* and *Mytilus*. Fish Invest
2929
2930 1008 Ser 2 26.
2931
2932 1009 Ward, J.E., Shumway, S.E., 2004. Separating the grain from the chaff: particle selection
2933
2934 1010 in suspension-and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. 300, 83–130.
2935
2936 1011 Wikfors, G.H., Ferris, G.E., Smith, B.C., 1992. The relationship between gross
2937
2938 1012 biochemical composition of cultured algal foods and growth of the hard clam,
2939
2940 1013 *Mercenaria mercenaria* (L.). Aquaculture 108, 135–154.
2941
2942 1014 Worrall, C.M., Widdows, J., 1983. Physiological changes following transplantation of the
2943
2944 1015 bivalve *Scrobicularia plana* between three populations. Mar. Ecol. Prog. Ser.
2945
2946 1016 Oldendorf 12, 281–287.
2947
2948
2949
2950
2951
2952
2953
2954
2955
2956
2957
2958
2959
2960
2961
2962
2963
2964
2965
2966
2967
2968
2969
2970
2971
2972
2973
2974
2975
2976