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ORIGINAL PAPER

Food regime modulates physiological processes underlying size differentiation in juvenile intertidal mussels *Mytilus galloprovincialis*

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Abstract Feeding and growth experiments were conducted to test the hypothesis that physiological attributes responsible for size differentiation among bivalves likely vary with environmental conditions. Juvenile mussels (*Mytilus galloprovincialis*) were collected from an intertidal population in Biscay, Spain (43°24′42,462″N02°56′43,659″W), in January 2007 and 2009. These mussels were maintained in the laboratory under either restrictive or optimal feeding conditions until fast- and slow-growing individuals on each maintenance regime could be identified. After fast- and slow-growing individuals were identified, the components of energy balance responsible for such growth rate differences were measured. The analysis of physiological traits indicates that under optimal food supply conditions, the capacity to ingest and absorb food and associated costs of growth are the main factors underlying growth rate differences. The set of physiological differences changed when size differentiation took place under restrictive food conditions. Higher rates of absorption coupled with reduced rates of metabolism accounted for faster growth in this case, especially under low food rations.

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Introduction

The energetic basis of interindividual differences in growth rate has been analysed in several bivalve species, and the somewhat conflicting results were systematized by Bayne (1999) in the form of physiological models accounting for growth differentiation. Discordant conclusions emerging from these studies suggest that differential growth potential is a consequence of multiple determinants. Accordingly, different combinations of physiological growth components have been reported to explain such endogenous differences. Two different combinations of these models have been used to describe the main trends reported in previous studies:

1. Fast growth resulting from higher rates of ingestion and absorption (*acquisition model*), usually coupled to reduced metabolic costs of growth (sensu Parry 1983) (*metabolic efficiency model*) (e.g. Holey and Foltz 1987; Toro and Vergara 1998; Bayne et al. 1999a, b; Bayne 2000; Pace et al. 2006; Tamayo et al. 2011, 2013, 2014, 2015).
2. Endogenous differences in standard metabolic requirements (*allocation model*) and metabolic costs of growth (*metabolic efficiency model*) as key factors explaining differences among specimens selected for fast growth (e.g. Garton et al. 1984; Hawkins 1995; Hawkins and Day 1996).

Overall, fast growth can depend on two different causes: 1) the ability to exploit available food sources by processing larger amounts of food more efficiently (combination 1), even though this strategy may lead to higher net metabolic outputs when growth rate differences are maximized

(Tamayo et al. 2011, 2013, 2014, 2015), or 2) the capacity to save higher amounts of energy due to reduced metabolic rates (combination 2).

A high degree of multi-locus heterozygosity is an endogenous factor widely correlated with enhanced growth rates among bivalves. Variable levels of heterozygosity appear to underlie the physiological traits responsible for fast growth in combination 1 (Holey and Foltz 1987; Toro and Vergara 1998; Pace et al. 2006; Meyer and Manahan 2010) and in combination 2 (Garton et al. 1984; Hawkins et al. 1986; Hawkins 1995; Bayne and Hawkins 1997). Although heterozygosity–growth associations in bivalves are widely accepted (see reviews: David 1998; Launey and Hedgecock 2001; Meyer and Manahan 2010), correlations reported in the literature seem intrinsically weak (reviewed by David 1998) and frequently explain 20–30 % of observed variance, at the most (Garton 1984; Koehn and Gaffney 1984; Diehl and Koehn 1985; Gentili and Beaumont 1988; Koehn et al. 1988; Alvarez et al. 1989; David et al. 1995). Increased multi-locus heterozygosity enhances the energy budget of fast growers through improved efficiency of protein deposition by reducing protein turnover rates. This mechanism leads to more efficient standard and routine metabolic rates (Hawkins et al. 1986, 1989; Bayne and Hawkins 1997).

Interpreting those combinations would benefit by relating the environment to the physiological characteristics resulting in faster growth. In this respect, food conditioning seems to affect the association between genetic and physiological traits (Bayne and Hawkins 1997), lending weight to the argument that benefits of a reduced protein turnover are likely dependent on available food levels. As stated by Bayne and Hawkins (1997), genetic factors promoting differential protein turnover requirements become relevant when food is abundant (when energy incorporation dominates energy balance), whereas heterozygosity and growth rate are uncorrelated at low food rations (when maintenance requirements are the determinant).

The aim of the present study was to test the hypothesis that the physiological components acting on size differentiation of fast- versus slow-growing individuals differ as a function of nutritional conditions during the selective phase. For this purpose, juvenile *Mytilus galloprovincialis* collected from a natural population were maintained in the laboratory until size differentiation occurred under two different experimental conditions: low and intermittent phytoplankton supply involving restrictive feeding conditions (Experiment 1, Expt. 1), and high and continuous phytoplankton ration involving optimal feeding conditions (Experiment 2, Expt. 2). In both experiments, individual with lowest and highest growth rates were selected and the physiological components of their energy balances determined at different food rations. The goal was to identify the specific physiological mechanisms involved in size differentiation and to compare these mechanisms among the different conditions applied to the selection of growth groups.

Materials and methods

Collection, maintenance and selection of fast (F)- and slow (S)-growing mussels

In both Expt. 1 and Expt. 2, juvenile (< 16 mm shell length, SL) mussels (*Mytilus galloprovincialis*) were collected from the rocky intertidal in the Plentzia estuary in Biscay, Spain (43° 24′ 42,462″ N 02° 56′ 43,659″ W).

Expt. 1: restrictive feeding conditions

Two hundred freshly collected mussels (November 16, 2007), 7–16 mm SL umbo to margin, were measured to the nearest 0.01 mm with a calliper and distributed in nine 1 mm SL groups (i.e. 7.00–7.99 mm, 8.00–8.99 mm and so on). These groups were maintained separately for 5 months with a low ration of phytoplankton (*Isochrysis galbana*, T-ISO, given at a packed volume concentration of particles of ≈0.5 mm^3 L^{-1}; equivalent to 0.22 mg POM L^{-1}) in a common feeding tank inside a recirculating sea water system regulated at 14 °C (environmental temperature at time of collection) and 34.0 salinity. In order to simulate the discontinuous feeding imposed by the tidal regime at the collection site (about 50 % emersion), mussels were fed phytoplankton concentrations stated above for 12 h day^{-1}. During the maintenance period, SL of individuals in each size group was measured on nine occasions.

At the end of the maintenance period, F and S mussels were selected by choosing the smallest and largest individuals (~P_{25} and P_{75} percentiles) in each size group. Individual growth rates were estimated as a daily increment (%) using the following formula:

$$100 \times ([\text{final SL} - \text{initial SL}]/\text{initial SL})/\text{t (day)}$$

where *final SL* is the average SL after 5 months of maintenance, and *initial SL* is the average SL of their size group at the beginning. F and S groups used in physiological experiments consisted of 30 fast- and 30 slow-growing mussels selected following this methodology. These individuals were between 12.25–19.29 mm SL in the F group and 10.70–16.17 mm SL in the S group.

Expt. 2: optimal feeding conditions

In this experiment, 200 mussels (March 19, 2009), 10–15 mm SL, were tagged for individual growth rate

Table 1 Characteristics of experimental diets used in physiological determinations in Expt. 1 and 2: *TPM* total particulate matter (mg L^{-1}), *PIM* particulate inorganic matter (mg L^{-1}), *POM* particulate organic matter (mg L^{-1}), *OC* organic content (decimal), *Vol.* packed volume (mm^3 L^{-1}), *n* number of samples

Experiment	Ration	TPM	PIM	POM	OC	Vol. (mm^3 L^{-1})	*n*
1							
Restrictive feeding condition	Low (RL)	0.73 ± 0.07	0.06 ± 0.04	0.67 ± 0.04	0.92 ± 0.05	1.59 ± 0.11	5
	High (RH)	1.32 ± 0.08	0.13 ± 0.05	1.19 ± 0.05	0.9 ± 0.03	3.2 ± 0.13	8
2							
Optimal feeding condition	Low (OL)	0.59 ± 0.18	0.14 ± 0.04	0.45 ± 0.18	0.75 ± 0.07	0.79 ± 0.17	11
	High (OH)	1.69 ± 0.25	0.52 ± 0.09	1.17 ± 0.18	0.7 ± 0.03	3.07 ± 0.32	9

Values are mean ± SD

measurements. Mussels were fed a high ration of phytoplankton cells (*Isochrysis galbana*, T-ISO, at a packed volume particle concentration of ≈3 mm^3 L^{-1}; equivalent to 1.2 mg POM L^{-1}), supplied continuously for 42 days at 14 °C. This food ration is similar to reported optimal rations for mussels in terms of maximum growth efficiency (Thompson and Bayne 1974). Compared to the conditioning ration in Expt. 1, this ration was around 10.5 times higher considering a 5.25-fold increase in food concentration and twofold increase in dosing time (24 vs. 12 h day^{-1}).

During the maintenance period, individual SL and live weight were recorded on six occasions to determine growth rate. Mussels were ranked according to growth rates in terms of both live weight and SL, and each individual was scored using a combination of points reflecting the rankings by both measures. The 20 highest and lowest scores were selected to form groups of F and S individuals. This procedure ensured that the selected mussels were those expressing the highest and lowest rates of increase in both SL and wet weight. Mussels selected in this way were 20.81–25.77 mm SL for the F group and 13.07–21.55 mm SL for the S group; their wet weights were 1.067–1.911 g for group F and 0.340–1.143 g for group S.

Physiological experiments with selected F and S mussels

Selected F and S juveniles were subsequently used in short-term feeding experiments. The F and S groups obtained in both Expt. 1 (restrictive feeding conditions, R) and Expt. 2 (optimal feeding conditions, O) were divided into two groups that were fed, respectively, diets of low (L) and high (H) particle concentrations, in order to determine the physiological components of energy balance under different food rations. Thus, the combination of conditioning and measuring food regimes generated four different experimental conditions (as shown in Table 1) henceforth termed as RL and RH (for Expt. 1 conditioning) and OL and OH (for Expt. 2 conditioning).

Diet characteristics

Diets used in measurements of physiological energetics were prepared by mixing *Isochrysis galbana* (T-ISO) with freshly collected and sieved (<67 μm) silt. The silt was used as an inorganic tracer in measuring absorption efficiency, following Conover (1986). Concentrated stocks of the diets were dosed into the feeding tanks at stable particle concentrations (given as packed volume: mm^3 L^{-1}) that were frequently checked with a Coulter Multisizer 3. For further characterization of diets, samples of suspensions were filtered onto ashed, pre-weighted GF/C filters and processed for total (TPM: mg L^{-1}), inorganic (PIM: mg L^{-1}) and organic (POM: mg L^{-1}) particulate matter concentration according to standard procedures (e.g. Tamayo et al. 2011). Organic content (OC) was estimated as POM TPM^{-1}. These dietary characteristics are given in Table 1.

Physiological determinations

Energy acquisition

Gross energy acquisition corresponded to the organic ingestion rate (OIR: mg POM h^{-1}) expressed in energy equivalents using a conversion factor of 18.75 J mg POM^{-1} reported for *I. galbana* (Whyte 1987). OIR was computed as the product of clearance rate (CR: L h^{-1}) and POM concentration in the diet (mg POM L^{-1}). Net energy acquisition corresponded to the absorption rate (AR: J h^{-1}), which is the product of OIR and the absorption efficiency (AE: decimal units).

For CR and AE determinations, mussels were placed in a filtering device consisting of an inverted conical flask serving as a filtration chamber and suited for the simultaneous collection of faeces with minimal disturbance of mussels (Tamayo et al. 2011, 2013, 2014; see Tamayo et al. 2015 for further description). Water from a feeding tank containing the diet was re-circulated through the chambers by means of multichannel peristaltic pumps to produce flow rates resulting in a 15–25 % reduction in particle concentration.

These flow rates were adjusted to ensure reliability of CR measurements while precluding water re-circulation by the mussels before leaving the system, which would cause CR underestimation. The particulate suspension in the feeding tank was maintained at the target concentration for each ration by addition of the appropriate volume of particles from concentrated stocks of the corresponding diet, while concentration (mm^3 L^{-1}) was frequently checked with a Coulter Multisizer 3. A control chamber (without mussels) served to correct particle sedimentation.

Mussels were given a 3-day acclimation period in these chambers prior to measurements. During the fourth day, CR measurements with each chamber were replicated 5–6 times and faeces (for AE determinations) were collected on three occasions. Sample values represent the average of these replicates.

In Expt. 1 (RL and RH conditions), 5 samples (chambers) were used ($n = 5$) per condition and growth group, each composed of 2 or 3 individuals of similar size with similar growth rates. Physiological rates in this case were referred to the mean individual weight of the group. In Expt. 2 (OL and OH conditions), 10 individuals ($n = 10$) were used per condition and growth group. In both experiments, different F and S individuals were used to perform measurements at high and low rations.

Clearance rates (CR: L h^{-1}) were calculated according to Crisp (1971) with the following formula:

$$CR = (F/n) * ((Ci - Co)/Ci)$$

where F is the flow rate (L h^{-1}), n is the number of individuals per sample, and Ci and Co are the particle concentrations in the outflows of control and experimental chambers, respectively.

Absorption efficiencies (AE: decimal units) were determined following Conover (1966), by comparing the organic content of food samples taken from the feeding tanks and faeces collected from the bottom of the chambers.

Metabolic expenditure

Once CR and AE measurements were completed, mussels were transferred to respirometers (150-ml capacity) filled with filtered sea water and sealed with LDO oxygen probes (cQ40d), and oxygen consumption was computed from the decrease in oxygen concentration over time (1–2 h). Controls (chambers without mussels) were used to check the stability of oxygen concentration during the measurement period. These measurements corresponded to the routine metabolic rate (RMR: J h^{-1}). For standard metabolic rate (SMR: J h^{-1}), mussels were starved for 48 h before measuring their oxygen consumption again. The difference between resting and active levels of metabolism is commonly referred to as the scope for activity (see Bayne and Newell 1983, for discussion on using the concept in physiological energetics of bivalves). Regarding our experimental conditions for RMR and SMR, we have proposed (Tamayo et al. 2013) the more specific term MSFG (metabolic scope for feeding and growth) to denote the difference between the two rates.

Rates of oxygen consumption were converted into metabolic rates using an oxycaloric coefficient of 20.08 J mLO_2^{-1} (Gnaiger 1983).

Energy balance

Resulting energy balance was computed as the scope for growth (SFG: J h^{-1}), which represents the difference between absorption rate (AR: J h^{-1}) and routine metabolic rate (RMR: J h^{-1}).

Size standardization

After physiological determinations, mussels were dissected out of the shells for flesh dry weight (DW) determination. Clearance and metabolic rates were standardized to a common size of 50 mg DW, which represents the average dry weight of F and S mussels in Expt. 1 and 2, by using the following expression:

$$Y_{st} = (W_{std}/W_e)^b * Y_e$$

where Y_{st} and Y_e represent the standard and experimentally recorded rates, respectively, W_e the weight of the experimental mussels, W_{std} the standard weight and b the power value that scales physiological rates to body weight in this species, 0.583 for CR (Bayne and Hawkins 1997) and 0.724 for metabolism (Bayne et al. 1973).

Statistical procedures

Significant effects with respect to growth category (F vs. S) and food ration on standardized physiological parameters were tested using a two-factor analysis of variance (ANOVA). Significant differences between values within experiments were checked by means of t test comparisons. A three-factor analysis of variance (ANOVA) was performed to test significant differences among growth categories (F vs. S), food rations and experiments.

Only in the case of RL, RMR and SMR measurements were carried out on different individuals, and thus, MSFG was computed without an associated variance. With the aim of unifying the analysis of MSFG variation, significant differences in metabolic rates promoted by activity level (routine vs. standard) and growth category were tested with a repeated measures two-factor ANOVA for RH, OL and OH conditions, and a standard two-factor ANOVA for RL.

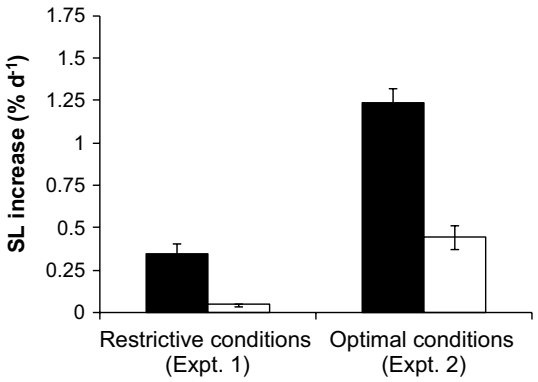

Fig. 1 Increase in SL (umbo to margin) (% day^{-1}) for F (*filled bar*) and S (*open bar*) *Mytilus galloprovincialis* selected under restrictive (Expt. 1) and optimal (Expt. 2) food regimes. Values are means ± 95 % C.I. ($n = 30$ and $n = 20$ for experiments 1 and 2, respectively)

Statistics were performed using SPSS 16, after homogeneity of variances was evaluated with the Levene test. Arc-sine transformation of ratios was performed when necessary according to the results of a normality test (Kolmogorov–Smirnov).

Results

Growth rates of mussels during size differentiation phase

Mean growth rates (% SL increment day^{-1}) of F and S juveniles selected under restrictive and optimal feeding conditions (Expt. 1 and 2, respectively) are shown in Fig. 1. Under restrictive feeding conditions, growth rate of F mussels was 0.35 ± 0.17 % day^{-1}, in contrast to 0.048 ± 0.025 % SL increment day^{-1} in slow growers (S). As for optimal feeding conditions, growth rate represented 1.24 ± 0.24 % and 0.442 ± 0.196 % SL increment day^{-1}, which corresponded to 4.36 ± 1.42 % and 1.26 ± 0.53 % weight increment day^{-1} in F and S groups, respectively. Condition of mussels was analysed by means of power equations relating DW (y) to SL (x), based on specimens used in physiological experiments. This relationship was: 0.024 $x^{2.62}$ ($n = 55$; r^2 0.61) and 0.026 $x^{2.64}$ ($n = 42$; r^2 0.86) for Experiments 1 and 2, respectively. In the case of Expt. 2, the relationship was 0.453 $x^{2.55}$ ($n = 21$; $r^2 = 0.75$) for F mussels and 0.251 $x^{2.77}$ ($n = 21$; $r^2 = 0.94$) in the case of S mussels. The similarity in exponents indicates an analogous condition of mussels across experiments and growth categories.

Physiological behaviour of F and S juveniles

Energy acquisition

Mean values for physiological components of energy balance of F and S juveniles selected under restrictive feeding conditions (Experiment 1) are given in Table 2, along with a two-factor ANOVA to test significance of effects related to juvenile type (F vs. S) and food ration (L vs. H). Concerning clearance rate (CR), growth category, food ration and an interaction term were significant in the ANOVA, indicating that F juveniles had a significantly higher CR than S juveniles at low ration and that a higher food ration promoted a significant reduction in CR in both growth groups, although it was greater in the F mussels (Fig. 2a). CR declined with increasing POM, apparently preventing the organic ingestion rate (OIR) from increasing above optimal values, and in fact, no significant effects of food ration on rates of ingestion were recorded (Table 2). Only juvenile growth type had significant effects on OIR, with F mussels having significantly higher values than S mussels at low rations (28 % higher).

Absorption efficiency only changed significantly with food ration, higher AEs being recorded with concentrated diets in both growth groups (Table 2). Combined responses of OIR and AE to the factors under study (growth category and food ration) would account for significant effects of both factors on rates of absorption (AR) (Table 2). F mussels had, at low and high POM concentrations, respectively, 19 and 9 % higher absorption rates than S juveniles.

Mean values for physiological components of growth measured in F and S mussels selected under optimal feeding conditions (Expt. 2), together with results of a two-factor ANOVA applied to these data for testing significant effects of growth category and food ration, are given in Table 3. Under optimal food conditioning, clearance rates of F and S mussels were not significantly different and were only affected by food ration (Fig. 2b). As in Expt. 1, clearance rate (CR) decreased with increasing POM (Fig. 2), but this reduction was not great enough to preclude the significant increase in organic ingestion rate (OIR) for both growth groups (ANOVA Table 3). In contrast, rates of ingestion were significantly higher in F mussels despite the fact that the CR differences between the groups were not significant (Fig. 2b). Differences in rates of ingestion were significant at high food concentration, with OIR 31 % higher in F than S mussels.

In Expt. 2, absorption efficiency was significantly affected by food ration (AE increased with increasing POM), whereas juvenile growth types were not significantly different (Table 3). Given that absorption rate (AR)

Table 2 Expt. 1. Physiological variables in fast (F)- and slow (S)-growing mussel juveniles selected in a restrictive food regime, tested at low and high rations

Physiological parameter	Growth category		Low ration		High ration	Summary of two-factor ANOVA		
						Growth category	Ration	Interaction
OIR (mg h^{-1})								
	F	a	0.288±0.022	a b	0.265±0.034	DF = 1	DF = 1	DF = 1
	S	b	0.224 ± 0.023	a b	0.250 ± 0.014	F = 9.21	F = 0.013	F = 3.57
						$p = 0.009$	$p = 0.909$	$p = 0.080$
AE (decimal)								
	F	b	0.369 ± 0.052	a	0.547 ± 0.052	DF = 1	DF = 1	DF = 1
	S	b	0.398 ± 0.025	a	0.532 ± 0.025	F = 0.082	F = 47.51	F = 0.932
						$p = 0.779$	$p = 0.000$	$p = 0.351$
AR (J h^{-1})								
	F	b c	1.979 ± 0.235	a	2.698 ± 0.361	DF = 1	DF = 1	DF = 1
	S	c	1.655 ± 0.087	a b	2.479 ± 0.025	F = 4.63	F = 37.44	F = 0.173
						$p = 0.049$	$p = 0.000$	$p = 0.684$
RMR (J h^{-1})								
	F	a	1.076 ± 0.131	a	1.151 ± 0.205	DF = 1	DF = 1	DF = 1
	S	a	0.949 ± 0.118	a	1.147 ± 0.366	F = 0.34	F = 1.50	F = 0.30
						$p = 0.566$	$p = 0.241$	$p = 0.590$
SFG (J h^{-1})								
	F	a b	0.903 ± 0.228	a	1.548 ± 0.402	DF = 1	DF = 1	DF = 1
	S	b	0.706 ± 0.180	a b	1.333 ± 0.389	F = 1.56	F = 14.88	F = 0.003
						$p = 0.232$	$p = 0.002$	$p = 0.957$
SMR (J h^{-1})								
	F	b	0.635 ± 0.116	a b	0.773 ± 0.180	DF = 1	DF = 1	DF = 1
	S	a b	0.767 ± 0.085	a	0.973 ± 0.150	F = 5.14	F = 5.54	F = 0.22
						$p = 0.040$	$p = 0.034$	$p = 0.649$
MSFG (J h^{-1})								
	F		0.440	a	0.378 ± 0.339			
	S		0.182	a	0.174 ± 0.466			

OIR organic ingestion rate, *AE* absorption efficiency, *AR* absorption rate, *RMR* routine metabolic rate, *SFG* scope for growth, *SMR* standard metabolic rate, *MSFG* metabolic scope for feeding and growth. Physiological rates were standardized to an equivalent 50 mg dry soft-body weight. Mean values are presented ± 95 % C.I. ($n = 5$ and 4 for F and S groups, respectively) together with a summary of two-factor ANOVA testing significant effects of growth category and experimental food ration. Different letters beside each mean value indicate significant differences among FL, SL, FH and SH (*t* test, $p < 0.05$)

was calculated as the product of OIR and AE, it was significantly affected by both growth category and food ration (ANOVA, Table 3). Thus, overall, F mussels had a higher AR than S juveniles even though their ration specific values did not differ significantly (10 and 27 % in OL and OH, respectively). Moreover, both groups increased their AR in a similar fashion with increasing food concentration (2.2 and 1.9 times for F and S, respectively).

A summary of a 3-factor ANOVA to test for significant effects promoted by growth category (F vs. S), food ration (L vs. H) and experiment (Expt. 1 vs. Expt. 2), upon physiological parameters of the energy balance, can be seen in Table 4. This analysis offered, in general terms, an interpretation of the results similar to those reported for the two experiments separately. Namely, F mussels had higher food processing capabilities that ultimately lead to higher absorption rates (AR), and ration increments enhanced energy acquisition. Interestingly, the factor termed experiment (i.e. Expt. 1 vs. 2) interacted significantly with food ration, likely due to the wider food range achieved in Expt. 2.

Metabolic expenditure

Mean values of metabolic rates under routine and standard conditions (RMR and SMR) are given in Table 2 and 3 for mussels conditioned to restrictive and optimal rations, respectively. Results of both experiments are compared (Fig. 3), with metabolic scope for feeding and growth

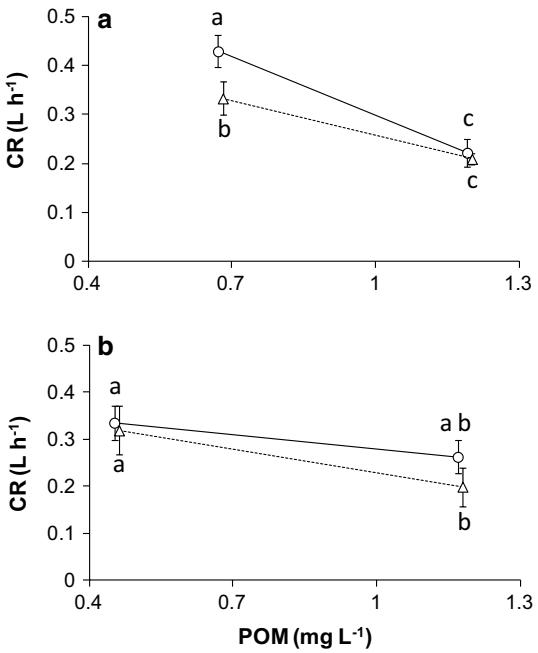

Fig. 2 Size standardized clearance rate (CR: L h^{-1}) as a function of particulate organic matter (POM: mg L^{-1}) for F (*circles*) and S (*triangles*) *Mytilus galloprovincialis* selected under **a** restrictive food regimes (Expt. 1, $n = 5$ and 4 for F and S groups, respectively); and **b** optimal food regimes (Expt. 2 $n = 8$ and 10 for F and S groups, respectively). Values are means ± 95 % C.I. CR was standardized to an equivalent 50 mg dry soft-body weight. Different letters indicate significant differences (*t* test)

(MSFG) values reported as a fraction of RMR. In Expt. 1, there were no significant effects on routine rates of growth category or ration. However, standard rates a) were higher in S when compared to F mussels and b) increased with the higher food ration in both types of mussels (Table 2). In Expt. 2, no significant effects were recorded on standard rates, while routine rates only differed significantly between low and high rations (Table 3). A 3-factor ANOVA (Table 4) confirmed that food ration was the only factor exerting a significant effect on RMR. Regarding the standard rate (SMR), both ration and the interaction between growth category and experiment exerted significant effects. These results account for the fact that, overall (1) food ration tends to increase SMR, and (2) while F individuals had similar SMRs in both experiments, S mussels had higher rates than F mussels in Expt. 1 and lower in Expt. 2.

With respect to metabolic scope for feeding and growth (MSFG), no differences were found between F and S mussels in Expt. 1 (RH rations) (Table 2). However, in Expt. 2 both growth category and ration were statistically significant. While higher rations caused a significant increase in MSFG for S mussels, similar values were recorded for F individuals (Table 3). Further statistical analysis of MSFG was done using four two-factor ANOVAs (see materials and methods). ANOVAs were performed in order to compare the four combinations of conditioning and experimental rations (RL, RH, OL and OH; Table 5). Tested factors were: 1) growth category (F vs. S) and 2) the level of metabolic activity (SMR vs. RMR). The latter factor was used to test whether metabolic differences between starved and fed conditions of mussels (in fact, MSFG) were statistically significant. Therefore, the interaction term in present analysis accounts for significant differences in MSFG between F and S mussels.

According to the ANOVA, feeding and growth activities promoted significant metabolic increments in comparison with starved conditions in all cases except for high ration in Expt. 1 (restrictive food conditioning, RH), where it was not statistically significant ($p = 0.095$; Table 5). However, the interaction between metabolic level and growth category was at the threshold of significance ($p = 0.05$) for this RH experimental condition, suggesting that metabolic behaviour during starvation differed between F and S mussels. In fact, a t test to compare metabolic levels within growth categories in this particular condition revealed significant differences between routine (RMR) and standard (SMR) metabolic rates for F mussels (*t* test, $t_8 = 2.781$; $p = 0.024$) and lack of significance for S mussels (*t* test, $t_6 = -0.578$; $p = 0.584$). Overall, analysis of factor interactions (Table 5) reveals some features about the metabolic behaviour of F and S mussels subjected to different food conditioning regimes. As a general rule, although the magnitude of MSFG differs between F and S mussels, the sign of this difference is strongly dependent on food conditioning: i.e. MSFG would be greater in F than in S mussels if maintained under restrictive food conditions and vice versa for F and S mussels conditioned to an optimal ration (Fig. 3). Strict interpretation of the ANOVA in Table 5 indicates, however, that this was significant only for metabolic measurements performed in mussels at low rations.

Scope for growth

SFG of mussels conditioned to restrictive food conditions was positively influenced by food concentration, effects of ration being significant (Table 2). Although consistently higher in F mussels, SFG differences between growth groups were not significant (Table 2); the benefits to fast growers of achieving significantly higher absorption rates (especially at low POM) were counterbalanced by higher metabolic expenditures.

Under optimal food conditioning, SFG was significantly affected by both growth category and food ration (Table 3). In addition to a positive influence of POM, large differences between F and S mussels were recorded, reflecting the combined effects of a greater capacity of F mussels to

Table 3 Expt. 2. Physiological variables in fast (F)- and slow (S)-growing mussel juveniles selected in an optimal food regime, tested at low and high rations

Physiological parameter	Growth category		Low ration		High ration	Summary of two-factor ANOVA		
						Growth category	Ration	Interaction
OIR (mg h^{-1})								
	F	c	0.151 ± 0.016	a	0.307 ± 0.040	DF = 1	DF = 1	DF = 1
	S	c	0.144 ± 0.023	b	0.233 ± 0.048	F = 4.99	F = 44.76	F = 3.35
						$p = 0.032$	$p = 0.000$	$p = 0.077$
AE (decimal)								
	F	b c	0.542 ± 0.025	a b	0.594 ± 0.019	DF = 1	DF = 1	DF = 1
	S	c	0.520 ± 0.058	a	0.618 ± 0.015	F = 0.007	F = 16.23	F = 1.52
						$p = 0.935$	$p = 0.000$	$p = 0.226$
AR (J h^{-1})								
	F	b	1.529 ± 0.156	a	3.407 ± 0.454	DF = 1	DF = 1	DF = 1
	S	b	1.409 ± 0.263	a	2.685 ± 0.542	F = 4.25	F = 59.62	F = 2.17
						$p = 0.047$	$p = 0.000$	$p = 0.150$
RMR (J h^{-1})								
	F	b	0.837 ± 0.139	a	1.327 ± 0.243	DF = 1	DF = 1	DF = 1
	S	a b	1.044 ± 0.205	a	1.478 ± 0.259	F = 2.45	F = 16.36	F = 0.062
						$p = 0.127$	$p = 0.000$	$p = 0.805$
SFG (J h^{-1})								
	F	b c	0.692 ± 0.161	a	2.080 ± 0.401	DF = 1	DF = 1	DF = 1
	S	c	0.364 ± 0.265	b	1.207 ± 0.614	F = 7.76	F = 26.79	F = 1.60
						$p = 0.009$	$p = 0.000$	$p = 0.215$
SMR (J h^{-1})								
	F	a	0.687 ± 0.117	a	0.821 ± 0.159	DF = 1	DF = 1	DF = 1
	S	a	0.540 ± 0.126	a	0.692 ± 0.256	F = 2.177	F = 2.35	F = 0.10
						$p = 0.150$	$p = 1.135$	$p = 0.992$
MSFG (J h^{-1})								
	F	b	0.150 ± 0.147	a b	0.507 ± 0.328	DF = 1	DF = 1	DF = 1
	S	a b	0.504 ± 0.181	a	0.785 ± 0.231	F = 7.29	F = 7.43	F = 0.103
						$p = 0.011$	$p = 0.010$	$p = 0.750$

OIR organic ingestion rate, *AE* absorption efficiency, *AR* absorption rate, *RMR* routine metabolic rate, *SFG* scope for growth, *SMR* standard metabolic rate, *MSFG* metabolic scope for feeding and growth. Physiological rates were standardized to an equivalent 50 mg dry soft-body weight. Mean values are presented ± 95 % C.I. ($n = 8$ and 10 for F and S groups, respectively) together with a summary of two-factor ANOVA testing significant effects of growth category and experimental food ration. Different letters beside each mean value indicate significant differences among FL, SL, FH and SH (t test, $p < 0.05$)

absorb energy and reduce metabolic expenditure by having significantly lower costs associated with growth represented by MSFG (Table 5; Fig. 3).

Discussion

The present study used significantly size-differentiated mussels maintained under food abundance or food deprivation for sustained periods in the laboratory. Maintenance of mussels under conditions of high phytoplankton supply (Expt. 2) intensified the overall growth potential of organisms, thus forcing the emergence of large interindividual differences in growth and, consequently, allowing us to form groups with very different growth rates (approximately 3 times higher for fast growers: 1.24 ± 0.24 vs. 0.44 ± 0.19 % increment in SL day^{-1} for F and S mussels, respectively). In contrast, the low food conditions in Expt. 1 promoted low growth rates reducing the scope for size differentiation. Nevertheless, after 5 months, large size differences were attained and the two groups selected for the highest (F) and the lowest (S) growth rates achieved 0.35 ± 0.17 % and 0.05 ± 0.03 increment in SL day^{-1}, respectively.

Comparison of physiological parameters involved in the energy balance between the different growth groups in the

Table 4 Comparison between Expt. 1 and 2: Summary of three-factor ANOVA testing significant effects of growth category, measuring ration and experiments on physiological parameters

Physiological parameter	Source of variation	df	F	*p*	Significance
CR (L h^{-1})	Growth category (A)	1	7.96	0.007	**
	Measuring ration (B)	1	61.18	0.000	***
	Experiment (C)	1	1.40	0.242	n.s.
	A × B	1	0.30	0.589	n.s.
	A × C	1	0.18	0.670	n.s.
	B × C	1	4.25	0.045	*
	A × B × C	1	3.81	0.057	n.s.
OIR (mg h^{-1})	Growth category (A)	1	8.38	0.006	**
	Measuring ration (B)	1	19.77	0.000	***
	Experiment (C)	1	11.82	0.001	**
	A × B	1	0.10	0.756	n.s.
	A × C	1	0.00	0.968	n.s.
	B × C	1	18.81	0.000	***
	A × B × C	1	4.37	0.042	*
AE (decimal)	Growth category (A)	1	0.07	0.797	n.s.
	Measuring ration (B)	1	55.81	0.000	***
	Experiment (C)	1	47.88	0.000	***
	A × B	1	0.00	0.972	n.s.
	A × C	1	0.03	0.874	n.s.
	B × C	1	6.88	0.012	*
	A × B × C	1	2.10	0.154	n.s.
AR (J h^{-1})	Growth category (A)	1	5.09	0.029	*
	Measuring ration (B)	1	58.50	0.000	***
	Experiment (C)	1	0.13	0.724	n.s.
	A × B	1	0.65	0.423	n.s.
	A × C	1	0.24	0.628	n.s.
	B × C	1	6.87	0.012	*
	A × B × C	1	1.32	0.256	n.s.
RMR (J h^{-1})	Growth category (A)	1	0.39	0.535	n.s.
	Measuring ration (B)	1	10.89	0.002	**
	Experiment (C)	1	1.01	0.321	n.s.
	A × B	1	0.03	0.856	n.s.
	A × C	1	1.82	0.184	n.s.
	B × C	1	3.22	0.079	n.s.
	A × B × C	1	0.25	0.622	n.s.
SFG (J h^{-1})	Growth category (A)	1	5.95	0.019	*
	Measuring ration (B)	1	28.05	0.000	***
	Experiment (C)	1	0.05	0.826	n.s.
	A × B	1	0.72	0.399	n.s.
	A × C	1	1.42	0.239	n.s.
	B × C	1	2.11	0.153	n.s.
	A × B × C	1	0.64	0.429	n.s.
SMR (J h^{-1})	Growth category (A)	1	0.04	0.846	n.s.
	Measuring ration (B)	1	4.82	0.033	*
	Experiment (C)	1	2.02	0.162	n.s.
	A × B	1	0.09	0.765	n.s.
	A × C	1	4.47	0.040	*
	B × C	1	0.04	0.841	n.s.
	A × B × C	1	0.03	0.863	n.s.

CR clearance rate (L h^{-1}), *OIR* organic ingestion rate (mg h^{-1}), *AE* absorption efficiency (decimal units), *AR* absorption rate (J h^{-1}), *RMR* routine metabolic rate (J h^{-1}), *SFG* scope for growth (J h^{-1}), *SMR* standard metabolic rate (J h^{-1}). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; *n.s.* non-significant differences

contrasting scenarios represented by Experiments 1 and 2 will be discussed with respect to the hypothesis in the Introduction, 'that the physiological components of growth acting in size differentiation of fast- versus slow-growing individuals would differ as a function of nutritional conditions occurring during the selective phase'.

In the presence of abundant food (Expt. 2), two physiological processes in fast and slow growers differed significantly: a) the capacity to ingest and absorb food and b) the magnitude of metabolic expenditures in excess of the standard rate, considered to represent the metabolic scope for feeding and growth. In accordance with the assumed use of this metabolic fraction, MSFG (= RMR-SMR) has been reported to be proportional to absorption and growth rates of mussels (*M. edulis*) measured in a wide range of food concentrations, thus representing an increasing fraction (26–45 %) of total metabolic expenses in a ~twofold range of SFG (Bayne et al. 1989). There is a good agreement between these and the increase in MSFG from low to high food rations (32–45 % on average) observed in the present study. However, this metabolic component also represents a significantly variable fraction of total expenses between growth groups (i.e. 27 and 50 % of RMR, on average, for F and S mussels, respectively) (Table 3; Fig. 3b).

In summary, mussels that grew rapidly in an abundant food supply combined an inherently higher capacity to acquire food, which was especially evident at high food rations (*acquisition* model) and energetically more efficient food absorption, assimilation and incorporation into tissues (*metabolic efficiency* model). These findings are similar to

those in our previous studies on hatchery-selected juvenile clams, *Ruditapes philippinarum* (Tamayo et al. 2011, 2013, 2015), and oysters, *Crassostrea gigas* (Tamayo et al. 2014).

Furthermore, and especially within the Ostreidae, similar results have been reported for several different species. It is noteworthy that these studies have been mainly performed with individuals selected for their variable growth and covering life stages from larvae to adults (Toro and Vergara 1998; Bayne et al. 1999a, b; Bayne 2000; Pace et al. 2006). This suggests that the physiological characteristics described above could represent a general tendency in bivalves when large growth rate differences are analysed.

In contrast, size-differentiated individuals did not exhibit the same set of physiological differences after 5 months under poor feeding conditions (Expt. 1). Primarily, selected F and S mussels did not differ significantly in SFG at the two food rations. Such a result is consistent with the weak size differentiation achieved during the 5-month period of maintenance at low food conditions and likely reflects the fact that the RL ration was two to three times higher than the maintenance ration in which mussels were allowed to size differentiate. Although mean SFG was lower in S than F mussels (especially at low ration), the difference was not significant (Table 2). In spite of that, F and S mussels differed significantly in CR and, hence, in ingestion and absorption rates, indicating that fast growers were better equipped for food acquisition than slow growers. However, CR differences between F and S juveniles were maximized at low POM concentration, and higher food rations cancelled out significant differences in CR. In other words, F juveniles would benefit from a higher capacity for food acquisition only at low food concentrations, i.e. in conditions where reduced energy incomes tend to magnify the contribution of reduced metabolic expenditure to total energy flux through the organism.

Another contrasting feature in comparing physiological behaviour of mussels maintained under restrictive and optimal feeding conditions concerns the metabolic aspects. Unlike the mussels in Expt. 2, MSFG in F mussels in Expt. 1 was higher than in S mussels in the same experiment. Since no significant changes in total metabolism (RMR) were observed either between F and S groups or between different food rations, it seems clear that differences in metabolic scope in this case were a consequence of reduced rates of resting metabolism (SMR) in fast growers (Table 2; Fig. 3a). Considering the significance values of the interactions in Table 5, the differences between growth groups were especially relevant when measured at low ration—as exemplified by the 46 % decline between RMR and SMR in F mussels (from 1.07 ± 0.13 to 0.63 ± 0.11) compared to the 20 % decline in S mussels (from 0.94 ± 0.11 to 0.76 ± 0.08)—even though RMR and SMR were barely

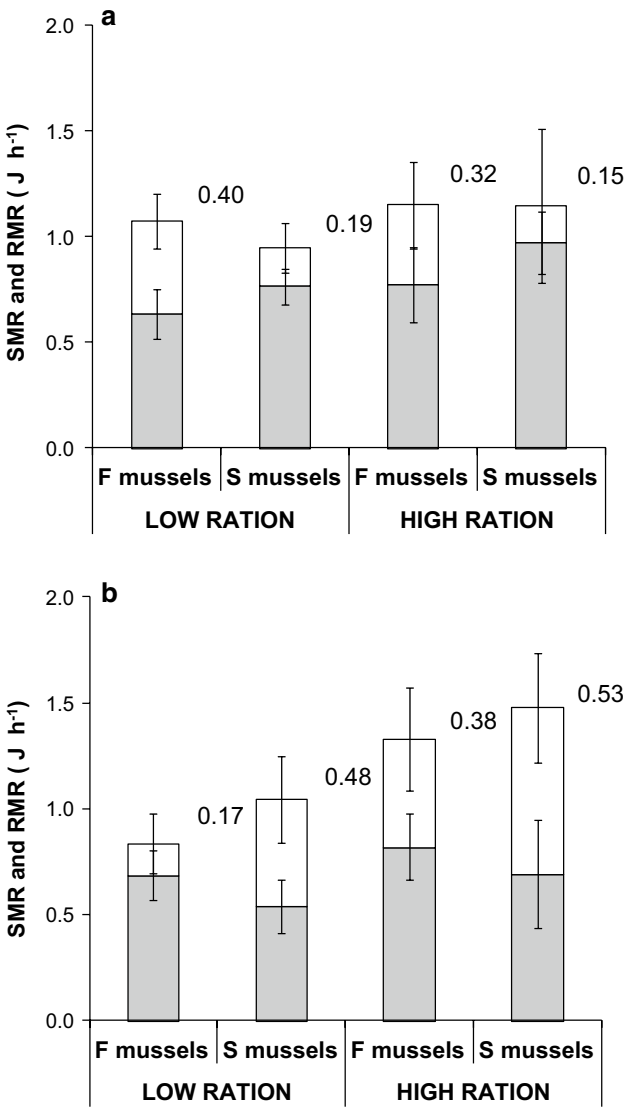

Fig. 3 Size standardized standard metabolic rate (SMR: J h^{-1}; *grey bar*) and routine metabolic rate (RMR: J h^{-1}; *full bar*) of F and S *Mytilus galloprovincialis* recorded at high and low rations. Numbers beside each bar correspond to the ratio of MSFG (*open bar*) to RMR = (SMR–RMR)/RMR. Mussels were selected under **a** restrictive food regimes (Expt. 1, $n = 5$ and 4 for F and S groups, respectively); and **b** optimal food regimes (Expt. 2 $n = 8$ and 10 for F and S groups, respectively). Values are means ± 95 % C.I. Metabolic rates were standardized to an equivalent 50 mg dry soft-body weight

significant ($p = 0.05$). Therefore, results point towards the existence of differences between fast and slow growers in their capacity to reduce metabolic expenditure under starvation. Unlike the mussels conditioned to an optimal ration, the fast growers under food limitation did not have an increased efficiency of food processing and growth, but their maintenance processes came at a reduced cost as shown by lower SMRs. This feature has previously been correlated with higher, although negative, SFG in mussels

Table 5 Summary of two-factor ANOVA testing for significant effects of a) level of activity (routine vs. standard) and, b) growth category (F vs. S) on metabolic rates in mussels from the four combinations of experimental conditions (*RL*, selected under restrictive food conditions and fed low rations; *RH*, selected under restrictive food conditions and fed high rations; *OL*, selected under optimal food conditions and fed low rations; *OH*, selected under optimal food conditions and fed high rations)

Experimental condition	Source of variation	DF	F	*p*
R L	Level of activity	1	26.550	0.000
	Growth category	1	0.001	0.971
	Interaction	1	4.580	0.050
R H	Level of activity	1	3.710	0.095
	Growth category	1	1.240	0.303
	Interaction	1	0.506	0.500
O L	Level of activity	1	28.030	0.000
	Growth category	1	0.101	0.755
	Interaction	1	8.220	0.011
O H	Level of activity	1	42.130	0.000
	Growth category	1	0.006	0.940
	Interaction	1	1.960	0.181

For RL conditions, independent groups of mussels were used for metabolic determinations; thus, a standard ANOVA was performed. For the rest of experimental conditions (RH, OL and OH), we performed an ANOVA of repeated measures, as the same groups of mussels were used in routine and standard measurements

under restrictive food levels (Hawkins 1995; Hawkins and Day 1996).

Our study shows that food conditions during the size differentiation phase might potentially alter the set of physiological variables underlying differential growth potential. This fact, that feeding conditions can affect the correlations between endogenous (genetic) factors and physiological rates, was previously suggested by Bayne and Hawkins (1997). These authors analysed possible correlations between growth rate and multilocus heterozygosity in small mussels that were sequentially held at increasing food levels. Increasing heterozygosity levels led to higher efficiencies of protein deposition and, as a consequence, lower costs of growth, thus allowing more heterozygous individuals to achieve higher growth rates. These series of correlations served to highlight the functional link between protein turnover rate, metabolic efficiency and genetic factors. However, Bayne and Hawkins (1997) also showed that significant correlations between heterozygosity and growth rate were only found at high food rations, while at lower rations, corresponding to maintenance conditions (low SFG), no significant correlations were found. It follows that genetic factors promoting differential protein turnover requirements become relevant under conditions of food abundance (i.e. when energy incorporation dominates the energy balance), whereas lack of correlation between heterozygosity and growth rate at low rations suggests that heterozygosity level does not modulate standard metabolic expenditure. Therefore, it is very likely that interindividual differences in SMR derive from causes other than differences in protein deposition efficiencies. In this respect, Pernet et al. (2008) reported that intra-specific differences in SMR are related to the unsaturation index of membrane fatty acids in *Crassostrea virginica*. These authors reported that both SMR and membrane unsaturation were lower in fast-growing hatchery oysters compared with slow-growing wild oysters. The higher unsaturation index in the latter corresponded mainly with higher 22:6n-3 levels whose importance in metabolic rate regulation of animals had already been observed (reviewed by Hulbert and Else 2005). Wild oysters had higher SMRs even though they were much more heterozygous than fast-growing hatchery oysters with lower SMRs and higher CRs.

Very likely the possession of a reduced maintenance metabolic output improves the probability of surviving under stressful conditions. Diehl et al. (1986) grew juvenile *Mytilus edulis* in a tidal salt marsh for 72 days and then starved them in the laboratory for 2 months. Recorded interindividual variability in rates of oxygen consumption suggested that reduced rates of metabolism were associated with increased resistance to weight loss during starvation rather than with the capacity for weight gain during feeding. An elegant experiment by LeBlanc et al. (2008) is even more relevant. Juveniles of *Mytilus edulis* were submitted to stressful conditions consisting in 11 h of air exposure followed by 6 h of exposure to 32° C water, which caused high mussel mortality. Survivors were used to determine SFG and multilocus heterozygosity and compared with control mussels. Higher heterozygosity in survivors than controls indicated that heterozygotes were somehow more resistant to stress. Measurements of SFG resulted in non-significant differences between treated and control mussels, but, meaningfully, there were significant differences in both RMR and SMR which were, respectively, 40 and 30 % lower for survivors than for control mussels.

A pertinent conclusion is that size differentiation of juvenile mussels in the natural environment results from a balance between opposing forces. These include a) periods of good trophic conditions characterized by high POM concentrations and long immersion times that would maximize growth rates of individuals well equipped to acquire food and process it at low costs, in spite of their relatively high resting metabolic rates, and b) periods of restricted trophic conditions of both low POM, low organic content of food and/or prolonged emersion times that would have a more adverse impact on those individuals unable to down-regulate their SMRs. This dual impact of feeding conditions upon the physiological profiles of individuals that are likely to grow faster might help understanding contradictory

conclusions in previous bivalve studies regarding the prevalence of energy input or output as the processes responsible for size differentiation.

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