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## Physiological basis of extreme growth rate differences in the spat of oyster (*Crassostrea gigas*)

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### Abstract:

Juvenile oysters (*Crassostrea gigas*) (produced in November 2009) reared under uniform hatchery conditions for 4 months were selected for extreme growth rate differences by repeatedly taking larger and smaller individuals to achieve weight differences >30× between fast (F) and slow (S) growers. The physiological basis of differential growth was analyzed in experiments in June 2010, where components of energy gain (clearance and ingestion rates and absorption efficiency), energy loss (metabolic rates) and resulting scope for growth ( $J h^{-1}$ ) were compared for groups of F and S oysters fed three different ration levels ( $\approx 0.5$ , 1.5 and 3.0 mg of total particulate matter  $L^{-1}$ ). In both F and S oysters, a higher food ration promoted asymptotic increases in energy gain rates through regulatory adjustments to clearance rates, which maintained similar absorption efficiencies across the food concentrations. No significant differences were found between growth groups in mass-specific physiological rates (i.e., per unit of body mass). However, the scaling of these rates to a common size in both groups using allometric coefficients derived for *C. gigas* revealed higher energy gain rates coupled with lower metabolic costs of growth in fast growers. Thus, appropriate size-standardization is essential in accounting for observed differences in growth rate. Present results are in accordance with previous reports on other bivalve species on the physiological processes underlying endogenous growth differences, suggesting that the same interpretation can be applied to the extremes of these differences.

## 1. Introduction

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Growth rates vary among individuals in natural bivalve populations (Mallet and Haley 1983; Dickie et al. 1984; Brown 1988) even when exposed to similar environmental conditions. This indicates the existence of inter-individual differences in both the capacity to acquire energy and its allocation to growth. Heritability of such inter-individual differences provides scope for improvement of bivalve production through the implementation of selection programs (Newkirk 1980; Sheridan 1997; Evans and Langdon 2006). Thus, understanding the energetics underlying inter-individual growth variability is essential in aquaculture.

Many of the early conclusions regarding the physiological determinants of inter-individual growth differences in bivalves were obtained by performing experiments with specimens sampled from natural populations (Garton et al. 1984; Gaffney et al. 1990; Hawkins et al. 1989a, 1989b), where local gradients in environmental factors, such as food or oxygen availability, might interact with endogenous factors in producing growth differences among individuals. Such interactions are not likely to occur in the case of sibling specimens produced in hatcheries through controlled spawning events and maintained in common culture conditions throughout their life-time. Research performed with specimens of this origin has shown that growth differences 1) are endogenously determined, meaning that they are not reversed by compensatory overfeeding of slow growers (Tamayo et al. 2011); 2) are evident from the very early larval stages (Pace et al. 2006; Hedgecock et al. 2007; Meyer and Manahan 2010); 3) persist through adulthood and are heritable, thus allowing selection to improve stock production (Bayne 1999, 2000; Bayne et al. 1999b); and 4) have been observed to be linked with physiological characteristics of energy acquisition and metabolic output (Garton et al. 1984; Hawkins et al. 1986, 1996; Hawkins 1995; Bayne and Hawkins 1997; Toro and Vergara 1998; Bayne 1999, 2000, 2004; Bayne et al. 1999a, b; Pace et al. 2006; Tamayo et al. 2011, 2013).

Intensive work has analyzed the differences in genotype and/or genetic expression underlying inter-individual differences in physiological performance. An early genetic approach based on multiple locus heterozygosity analysis used by many researchers (Kohen and Gaffney 1984; Hawkins et al. 1986; Hawkins 1995; Hedgecock et al. 1995; Bayne and Hawkins 1997; Myrand et al. 2009) recognized an advantageous hybrid vigor: more heterozygous individuals tended to grow faster due to their diminished metabolic demand (Garton et al. 1984; Hawkins 1995) which resulted from more efficient protein turnover (Hawkins et al. 1986, 1989a; Hawkins and Day 1996; Bayne and Hawkins 1997; Morgan et al. 2000). Recently, new techniques for massive DNA sequencing have been widely applied to the study of bivalves (Hedgecock et al. 2007; Jenny et al. 2007; Tanguy et al. 2008; Meyer and Manahan 2010; Zhang et al. 2012; Zhao et al. 2012; Genard et al. 2013) and can help link physiological performance to quantitative or qualitative differences in genotype or gene transcription.

Several species of oysters in the family Ostreidae have been the subjects of recent studies on the physiological basis of inter-individual differences in growth rates. Comparison of the physiological performance of fast versus slow growers has been undertaken in larvae (Pace et al. 2006), juveniles (Toro and Vergara 1998; Pernet et al. 2008) and adults (Toro et al. 1996; Bayne 1999, 2000, 2004; Bayne et al. 1999a, b).

These studies reported significant differences between fast and slow growers in energy acquisition rate (i.e. differences in filtering activity and, correspondingly, in food ingestion and absorption rates) (Toro and Vergara 1998; Bayne et al. 1999a, b; Bayne, 1999, 2000; Pace et al. 2006; Pernet et al. 2008), as well as in the rate of energy loss

through respiration (metabolic rate). Following Bayne's terminology(1999), reported differences have been found to fit both the energy allocation model (i.e. different patterns of energy allocation between maintenance and growth, Bayne 1999, 2000; Pernet et al. 2008) and metabolic efficiency model (i.e. different energy costs of growth, these defined as the amount of energy expended per unit of tissue growth: Toro and Vergara 1998; Bayne et al. 1999a, b; Bayne 1999, 2000; Pace et al. 2006; Pernet et al. 2008).

In the present study, spat of the oyster *Crassostrea gigas* belonging to a single cohort produced in a hatchery were subject to successive selection of size extremes to achieve two groups with an ~ 30x difference in mean size. Aims were to compare physiological measurements used in determining scope for growth (SFG), as well as the digestive balance of dietary carbon and nitrogen in these two groups of fast (F) and slow (S) growers, in order to determine the physiological traits underlying these extreme differences in growth rate among individuals. Physiological measurements were performed in a range of dietary conditions to explore the possibility that fast and slow growers respond differently to changes in food availability.

## 2. Material and methods

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Oyster (*Crassostrea gigas*) spat were produced in a hatchery (November 2009), the Marine Culture Farm of TINAMENOR S.L. (Pesués, Cantabria, North Spain) through a massive spawning of some 100 adult oysters collected from a wild population on the rocky shore close to the farm. Larvae were cultivated at an initial density of 10-20 mL<sup>-1</sup> in three larval tanks. Two growth groups were segregated by successive selection of individuals chosen from the extremes of the size range. Feeding conditions consisted of a mixture of the algae *Isochrysis galbana* (T-ISO clone), *Chaetoceros muelleri* and *Tetraselmis suecica* supplied at a ration level representing 5% of live weight d<sup>-1</sup>.

Both groups fast (F) and slow (S) growers were delivered on February 25<sup>th</sup> 2010 and maintained in the laboratory for 3 months at 17°C under identical feeding conditions, consisting of a mixture of living *Isochrysis galbana* (T-ISO clone) and *Pheodactylum tricornutum* freeze-dried paste (purchased from Fitoplancton Marino) at a ratio of (7:3). No significant mortality (<1%) was recorded for either F or S oysters during the 3 months of maintenance in the laboratory. When physiological experiments were initiated in June, the sizes of F and S clams were 1955 ± 214.7 and 54 ± 2.6 mg live weight (24.80±0.95 and 6.08±0.90 mm shell length), respectively.

The experimental design included measurements of the physiological components of the energy balance in both groups of oysters maintained with three different food rations (see below). Additionally, diet and feces samples corresponding to the medium and high ration were collected for CHN analysis in order to compute digestive balances and absorption efficiencies for carbon and nitrogen. Physiological determinations with each diet regime took 6 days and were performed using different specimens.

### 2.1. Diet characteristics

Cultures of the alga *Isochrysis galbana* (T-ISO) had silt particles added to provide diets with an inorganic tracer for absorption efficiency determinations according Conover (1966). Concentrated stocks of these diets were dosed into the feeding tanks at rates set to provide stable particle concentrations and were checked frequently by monitoring

particulate volumes ( $\text{mm}^3 \text{L}^{-1}$ ) using a Coulter Multisizer 3. Target values for the different rations were:  $0.5 \text{ mm}^3 \text{L}^{-1}$  for the low ration,  $3.0 \text{ mm}^3 \text{L}^{-1}$  for the medium ration and  $6.0 \text{ mm}^3 \text{L}^{-1}$  for the high ration.

Characterizing food suspensions involved daily extraction of duplicate water samples from the feeding tanks later filtered through ashed pre-weighted GF/C glass-fiber filters. These filters were subsequently processed to determine concentrations of total particulate matter (TPM:  $\text{mg L}^{-1}$ ) and inorganic and organic particulate matter (PIM and POM:  $\text{mg L}^{-1}$ ). Salts retained in the filters were rinsed out with a solution of ammonium formate sea-water isotonic (0.9%; w:v), then filters were dried at  $110^\circ\text{C}$  for 24–48 h, weighed, ashed at  $450^\circ\text{C}$  (6 h) and weighed again. TPM and PIM were estimated, respectively, as the dry and ash weight increment of the filters and POM as the weight loss of this filtered material on ashing. Organic content (OC) was estimated as POM/TPM. Additionally, three samples of the stock diet taken at different points during the experiment were divided into three replicas and each one filtered through pre-ashed ( $450^\circ\text{C}$ ) GF/C filters for elemental (CNH) analysis. These filters were stored at  $-20^\circ\text{C}$  until combustion in a Euro EA elemental analyzer (EuroVector) which had been calibrated using acetanilide as standard. Dietary characteristics of the three different rations are shown in Table 1.

## 2.2. Physiological determinations

Due to the great size differences between F and S oysters, the number of individuals  $\text{sample}^{-1}$  in physiological determinations was chosen to achieve a common biomass: from a single individual in F oyster samples to 33 specimens  $\text{sample}^{-1}$  in S oysters. Diet treatments proceeded with replicated samples disposed in individual chambers suited for clearance rate and absorption efficiency determinations: oysters were placed on plastic grids attached to the upper part of inverted conical chambers to allow collection of feces with minimal disturbance (Tamayo et al. 2011, 2013). Water from a feeding tank containing the diet and regulated to  $17^\circ\text{C}$  was re-circulated through the chambers by means of a multichannel peristaltic pump regulated to produce flow rates required to achieve a 15–25% of reduction in particle concentration inside the chambers while the particulate suspension in the tank was maintained at the target concentration ( $\text{mm}^3 \text{L}^{-1}$ ) by the addition of an appropriate amount of particles delivered from a concentrated stock of the diet. A control chamber (without oysters) was used to correct for particle sedimentation. Oysters were allowed to accommodate for 24 h in these chambers which were cleaned prior to the start of measurements.

Clearance rates (CR:  $\text{L h}^{-1}$ ) of oysters were calculated, according to Crisp (1971) as:

$$\text{CR} = F/n * ((C_i - C_o) / C_i)$$

where F is the flow rate ( $\text{L h}^{-1}$ ),  $n$  is the number of specimens per chamber ( $n = 1$  for F oysters and  $n = 33$  for S oysters) and  $C_i$  and  $C_o$  are the particle concentrations in the outflows of control and experimental chambers, respectively. Each sample is represented by the average value of 6–7 determinations in a chamber over 48 h and reported values are means of 5 samples (chamber measurements) for both F and S oysters.

### 2.3. Ingestion rates

Organic ingestion rate (OIR:  $\text{mg h}^{-1}$ ) was computed as the product of CR and POM concentration ( $\text{mg POM L}^{-1}$ ) in the feeding tanks. Ingestion rates of carbon and nitrogen ( $\text{IR}_C$  and  $\text{IR}_N$  respectively) were calculated as the product of CR and particulate organic carbon (POC:  $\text{mg L}^{-1}$ ) and nitrogen (PON:  $\text{mg L}^{-1}$ ), respectively.

### 2.4. Absorption efficiencies and absorption rates

Samples of biodeposits collected from the bottoms of chambers were filtered onto ashed pre-weighted GF/C glass-fiber filters and processed for total and organic matter as indicated for food samples. In the case of medium and high rations, duplicate samples were filtered onto ashed GF/C filters, stored at  $-20^\circ\text{C}$  and processed for elemental (CNH) analysis.

Apparent absorption efficiency of organic matter (decimal units) was determined following Conover (1966), as:

$$AE = (f - e)/(1 - e)*f$$

f and e representing the organic contents of food and biodeposits, respectively.

Absorption rate (AR:  $\text{mg h}^{-1}$ ) of total organic matter was calculated as the product of OIR and AE. Carbon and nitrogen absorption rates ( $\text{AR}_C$ ,  $\text{AR}_N$ :  $\text{mg h}^{-1}$ ) were calculated as the balance between their ingestion and egestion rates, where the last was computed as total organic egestion rate [ $\text{OER} = \text{OIR} \times (1 - \text{AE})$ ] multiplied by the C or N content in the feces.

Carbon and nitrogen AE ( $\text{AE}_C$  and  $\text{AE}_N$  respectively) were calculated as the ratio between their absorption and ingestion rates. Reported values of AE are based on means of organic content or C and N content of biodeposits sampled from 5 chambers ( $n = 5$ ) for each growth group. For conversion of OIR and AR to energy units, an energy equivalent of  $18.75 \text{ J mg POM}^{-1}$  was applied based on reported data for *I. galbana* (Whyte 1987).

Metabolic rates ( $R$ :  $\text{J h}^{-1}$ ) were derived from measurements of oxygen consumption ( $\text{VO}_2$ :  $\text{mLO}_2 \text{ h}^{-1}$ ) using an oxycaloric coefficient of  $20.08 \text{ J mLO}_2^{-1}$  (Gnaiger 1983). Oysters were confined in 150-mL chambers ( $n = 5$  for each growth group) sealed with LDO oxygen probes connected to oxymeters (HATCH HQ40d) and rates of oxygen consumption estimated from the decrease in oxygen concentration over time (1–2 h). Controls (chambers without oysters) were used to check the stability of oxygen concentration during the measurement period.

Scope for growth (SFG:  $\text{J h}^{-1}$ ) was computed as the difference between absorption rate and metabolic rate (both in  $\text{J h}^{-1}$ ) which implies, in fact, a slight overestimation of the true SFG, energy losses due to ammonia excretion not having been considered (see Bayne and Newell 1983).

## 2.5. Expression of physiological results

Once physiological measurements were completed, individuals were processed for determination of tissue dry weight (TDW: mg). Soft tissues of F oysters were dissected and TDW was individually determined after drying the tissues at 80°C for 48 h. For S oysters, given the elevated number and the difficulty of dissecting small specimens, individual TDWs were estimated from total dry weights (totDW: mg) (obtained after drying at 80°C for 48 h), using the following equation that was obtained with a group of S oysters broadly covering the size range of experimental S individuals:

$$TDW = 0.0747 (\pm 0.008) \times \text{totDW} - 0.0467 (\pm 0.391), r^2 = 0.8315, F_{1,20} = 98.713, P < 0.000, n = 22$$

Following Tamayo et al (2013), physiological rates were expressed as: 1) mass-specific rates ( $Y_{\text{SPC}}$ , rate TDW unit) and 2) size-standardized rate ( $Y_{\text{STD}}$ ). Clearance and metabolic rates were standardized to common flesh dry weights ( $TDW_{\text{STD}}$ ) by using the expression:

$$Y_{\text{STD}} = (TDW_{\text{STD}} / W_{\text{EXP}})^b * Y_{\text{EXP}}$$

where  $Y_{\text{STD}}$  and  $Y_{\text{EXP}}$  represent the standard and experimentally recorded rates, respectively.  $W_{\text{EXP}}$  represents the weight of the experimental oysters and  $b$  the mass-exponent that scales physiological rates to body weight in this species ( $b = 0.439$  and  $0.8$  for CR and R, respectively, from Bougrier et al. 1995).

## 2.6. Statistical procedures:

Physiological measurements were compared for differences between or among treatments (growth categories and ration) by means of multiple factor ANOVA (using SPSS 11) after normality was tested (Kolmogorov-Smirnov) and homogeneity of variances evaluated by means of the Bartlett test (Zar 1984). When necessary, arc-sine transformation of ratios was performed. Slopes of the linear regressions for the costs of absorption between F and S oysters were compared using a slope comparison test (Zar 1984).

## 3. Results

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### 3.1. Mass-specific physiological components of energy balance

Mass-specific values of clearance rate ( $\text{mL h}^{-1} \text{g TDW}^{-1}$ ), ingestion rate of organic matter ( $\text{mg h}^{-1} \text{g TDW}^{-1}$ ), absorption rate ( $\text{J h}^{-1} \text{g TDW}^{-1}$ ), metabolic rate ( $\text{J h}^{-1} \text{g TDW}^{-1}$ ) and scope for growth ( $\text{J h}^{-1} \text{g TDW}^{-1}$ ) recorded for F and S oysters are shown (Table 2), where absorption efficiency (fraction) is also included. The table incorporates a summary of the 2-factor ANOVA used to test the effect of growth category and food ration.

While growth category had no significant effect on mass-specific physiological measurements, food ration had significant effects on all physiological variables:  $CR_{SPC}$  increased from low to medium rations but decreased from medium to high rations (Fig. 1a). Since CR directly determines rates of ingestion, this behavior results in ingestion rate regulation across variable amounts of suspended food. Indeed, data in Table 2 show rates of organic ingestion ( $OIR_{SPC}$ ) attained a nearly stable value for medium and high rations. Mean values for AE did not differ between F ( $0.652 \pm 0.058$ ) and S ( $0.676 \pm 0.033$ ) oysters but differed significantly between food rations. Significance of the interaction term (Table 2) accounts for the fact that maximal AE values were recorded with high and low rations in F ( $0.69 \pm 0.04$ ) and S ( $0.79 \pm 0.01$ ) oysters, respectively. Mass-specific absorption rates ( $AR_{SPC}$ ) increased significantly with rising POM (Table 2, Fig.1b), but at a decreasing rate caused by ingestion regulation. Metabolic expenditures ( $R_{SPC}$ ) also increased significantly with POM (Fig. 1c) and resulting mass-specific scope for growth ( $SFG_{SPC}$ ) values changed from negative values with the low ration to a plateau for medium and high rations (Fig. 2).

### 3.2. Absorption of C and N

Table 3 compares C:N ratios of medium and high ration diets and feces of F and S oysters fed by these two diets, showing also the corresponding values of absorption efficiency of C and N. Concerning C:N ratios of feces, no significant effect of growth category was recorded while the effect of ration appeared significant (Table 4). However, the latter can be accounted for by significant differences in diet composition between medium and high rations since their respective C:N indexes also differed significantly (ANOVA,  $F_{(1,4)} = 17.889, P = 0.013$ ). In accordance with the above result, no significant differences were recorded in the absorption efficiency for C and N (ANOVA,  $F_{(1,34)} = 1.425, P = 0.241$ ; for the comparison  $AE_C$  vs  $AE_N$ ). Food ration but not growth category exerted significant effects on both  $AE_C$  and  $AE_N$  (Table 4), these effects paralleling recorded behavior of overall AE for organics (Table 2).

### 3.3. Size-standardization of physiological components of the energy balance

Physiological measurements have been size standardized to a range of tissue dry weights (TDW: mg) for the full size-spectrum of oysters used in our experiments, using mass-exponents for clearance (and hence ingestion and absorption) and metabolic rates reported by Bougrier et al. (1995). The size-dependency of these rates standardized according to allometric rules is plotted (Fig. 3) for net energy intake ( $AR_{STD}$ ) and metabolic expenditure ( $R_{STD}$ ) recorded with the medium ration in both growth categories. The figure illustrates that higher values of  $SFG_{STD}$  (shaded region between the lines) for F oysters in the whole range of experimental sizes are the consequence of significantly higher values of  $AR_{STD}$  in spite of also having significantly higher  $R_{STD}$ . Namely, F:S ratios calculated for clearance, ingestion or absorption rates were around 5 (i.e., computed rates for F oysters were  $\sim 5x$  the rates of S seeds of the same size) while these ratios were only  $\sim 2$  when calculated for metabolic rates. As a result, the critical size at which null SFG is predicted ( $AR = R$ ) is increased  $\sim 30$  times (Fig. 3), nearly the estimated differences in growth rate between F and S oysters.

## 4. Discussion

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We have quantified the physiological components of the energy budget in oysters belonging to a single cohort that had more than 30x differences in their body size after being reared under the same environmental conditions. Such high inter-individual growth rate differences illustrate the profound impact that endogenous (genetic) factors can have on growth potential in oysters (Toro et al. 1996; Toro and Vergara 1998; Bayne et al. 1999a, b; Bayne 2000, 2004; Pace et al. 2006).

The magnitude of differences in growth rate between the individuals selected as fast and slow growers in the present study contrasts with those in previous experiments: we compared specimens from a single cohort differing ~ 36-fold in biomass (1950 vs 54 mg live weight approximately), while the range of differences between fast and slow growing oysters reported in the literature was 2 to 5-fold (Bayne et al. 1999a, b; Bayne 1999, 2000; Pace et al. 2006) (*Ostrea chilensis*: Toro et al. 1996; Toro and Vergara 1998) (*Crassostrea virginica*: Pernet et al. 2008). Our experimental approach was to improve the resolution of previous physiological comparisons by enhancing size differentiation between slow and fast growers, but this forces us to consider allometric size-scaling rules to interpret the results. Allometric relations are expressed as  $Y = a W^b$ ; where  $Y$  is the physiological rate,  $a$  is the proportionality constant,  $W$  is the weight and  $b$  is the mass-exponent scaling the physiological rate with body weight. From the extensive literature regarding allometric relations for CR and  $VO_2$  in bivalves (see Bayne and Newell 1983; Riisgård, 1988, 1998; Vladimirova et al. 2003; Ibarrola et al. 2008, 2012 for review) three conclusions can be drawn: 1) the elevation ( $a$ ) of the relationship is affected by exogenous factors (such as season and temperature) and, therefore, reflects the physiological status of the population; 2) mass-exponent ( $b$ ) is species-specific, not dependent on exogenous factors, and has values typically  $< 1$ , indicating that size-specific physiological rates decrease as the organism increases in size; and 3) mass-exponents for clearance rate are usually lower than those for oxygen consumption (0.4–0.6 for CR and 0.6–0.8 for  $VO_2$ ), indicating that size-specific reduction in filtering activity is more pronounced than size-specific reduction in metabolic rate. This factor explains the progressive decline in growth rate as the individual approaches its maximum size.

In the present experiments, the F and S spat had no significant differences in size-specific physiological rates (i.e. physiological rate divided by tissue dry weight) of both energy incorporation ( $CR_{SPC}$ ,  $OIR_{SPC}$  and  $AR_{SPC}$ , Table 3) and expenditure ( $R_{SPC}$ ). Thus, our results contradict allometric rules indicating either that F oysters have higher or S oysters lower physiological rates than those expected in consideration of their respective sizes. The magnitude of physiological differences between S and F spat can only be compared once the size-effect is corrected by standardization to a common body weight using allometric mass-exponents (see also Tamayo et al. 2013). It should be emphasized that in the particular case of *Crassostrea gigas*, mass-exponents for CR and  $VO_2$  were determined in an unusually accurate way by Bougrier et al. (1995) after measuring physiological rates of more than  $> 300$  individuals with a broadly range size (100–3000 mg TDW) at different water temperatures (5–32°C) and seasonal conditions (September and February). Mass-exponents obtained by Bougrier and collaborators have been broadly used for size-standardization of physiological rates in *Crassostrea gigas* (Barille et al. 1997; Bayne et al. 1999a, b; Ropert and Gouletquer 2000; Haure et al. 2003). In the present experiments, standardization of physiological results to different tissue dry weights (Fig. 3) revealed the following: 1) F oysters had a higher energy balance over the whole size range; 2) higher growth potential for F oysters is the result of a greater difference between values for  $AR_{STD}$  and  $R_{STD}$ ; for instance, standardization of rates of F spat to the body size (TDW: mg) of



S spat indicates that, after correction for the size-effect, fast growers are able to process five times more food at only twice the metabolic cost. Conversely, for S oysters, standardization of the rates to the equivalent TDW of F oysters indicates that such hypothetical individuals would have higher metabolic rates than AR and, hence, negative scope for growth. Indeed, results envisaged by allometric relationships indicates that the energy balance would become negative once the S oysters achieve an equivalent TDW of 5 mg and this prediction is consistent with the lack of growth of S oysters in the laboratory. Therefore, after proper size-standardization of results, it became evident that the higher growth potential displayed by F oysters was based on 1) higher filtering capacity and 2) lower energetic costs per unit of assimilated food than slow-growing oysters.

The coupling of high feeding rates with low costs of growth in faster growing oysters has been previously reported. In a series of studies using controlled crosses of inbred families of *C. gigas*, growth heterosis in juvenile oysters (Bayne et al. 1999a) and larvae (Pace et al. 2006) was found to be mainly based on the higher feeding rates and growth efficiencies in the hybrids. Indeed, average differences in growth rate (2 to 2.5-fold) between inbred lines and hybrids were sustained by CR differences of 1.2 to 2.5-fold, this factor explaining 50% of variance in growth in the case of larvae. On the other hand, lack of significant differences in size-specific metabolic rates suggested reduced costs of growth in faster growing hybrid larvae and was associated with increased efficiency of protein deposition (Pace et al. 2006). Both components of growth differentiation were also found in comparing adult (17 mo-old) *C. gigas* from two lines of different parentage where faster growing (5.9 vs 3.5 mg tissue dry weight d<sup>-1</sup>) resulted from higher feeding rates (x 1.56) coupled with a 42% reduction in costs of growth (Bayne 1999). Quite similar results have been reported for other species in the Ostreidae when comparing physiological behavior in fast-growing individuals from selected lines with that of unselected field-caught individuals in the cases of *Saccostrea commercialis* (Bayne et al. 1999b; Bayne 2000) and *Crassostrea virginica* (Pernet et al. 2008), or between differentially growing cohorts of *Ostrea chilensis* (Toro et al. 1996; Toro and Vergara 1998). Significantly, group differences in clearance rates and costs of growth in our present study exceed those reported earlier, suggesting that the same fundamental physiological factors accounting for inter-individual growth variability would apply to the extremes of the range of such variability.

Considering these factors, it is clear from previous reports that the main contribution to growth differentiation comes from the variable capacity to acquire energy and this raises two questions that, for the moment, have only been poorly investigated. The first one concerns the possible existence of morphological-functional determinants of feeding rate, such as the faster feeding contributing to higher growth rates in clams *Ruditapes philippinarum* that rely on larger gill areas (Tamayo et al. 2011). Genetic causes of differences in gill size are presently unknown. However, variable degrees of gill development among growth groups might be related to the negative correlation of growth rate with an aneuploidy index of gill cells reported in both oysters and clams (Thiriot-Quievreux et al. 1992; Zouros et al., 1996; Leitão et al. 2001; Batista et al. 2007; Teixeira de Sousa et al. 2011). This association between reduced growth and chromosome deletion has been interpreted on the basis that progressive haploidization of somatic cells would cause unmasking of deleterious recessive genes (Zouros et al. 1996; Launey and Hedgecock, 2001), which might result in deficient organ development.

No significant differences were found in the responses of S and F oysters to differences in food availability. Higher particle concentrations of high organic content resulted in the usual response in continuous feeders (see Riisgård et al. 2011 for a recent review): the increase in CR between the low and medium rations, and reduction

of filtration rate at the high particle concentration ( $6 \text{ mm}^3 \text{ L}^{-1}$ ), is a regulatory behavior enabling maintenance of AE relatively independent of food concentration in both F and S oysters. However, a small but significant difference was found in the trend exhibited by AE with CR in both groups: the increase in OIR from the low to medium ration promoted a reduction in AE, which was only significant for S oysters (Table 2). This differential behavior could be indicative of a relatively higher capacity for compensatory adjustment at the digestive level in F oysters. New aspects of digestive behavior, including the quantitative assessment of cycles of intracellular digestion, should be studied to answer this question, since in our previous experiments with the clam *R. philippinarum* (Tamayo et al. 2011) we failed to find significant differences between fast and slow growers either the size of the digestive glands (mg per standard size individual), their protein content (mg protein / mg dry weight) or specific cellulase activity (mg maltose / mg protein) in the digestive gland (unpubl data).

The second component in the physiological tandem accounting for growth differences between F and S oysters is the differential costs of growth. These costs have been estimated from linear regression analyses for routine rates of metabolism (R) vs absorption rates (AR) (Fig.4). Following Bayne (Bayne et al. 1999b; Bayne 2000) those regression equations can be resolved into two metabolic components of routine rates (Parry 1983): the standard requirements represented by rates of metabolism for AR=0 (intercepts), representing the metabolic demand in the absence of feeding and growth, and the costs of growth (slopes), representing the metabolic requirements per unit of food absorption and assimilation into tissue growth. For rates ( $R_{\text{STD}}$  and  $AR_{\text{STD}}$ ) standardized to an equivalent 2 mg TDW (the mean value of S oysters), regression equations for the lines plotted in Figure 4 were:

-F oysters:  $RR \text{ (J h}^{-1}\text{)} = 0.179 (\pm 0.032) \times AR \text{ (J h}^{-1}\text{)} + 0.055 (\pm 0.030)$ ,  $r^2 = 0.7589$ ,  $F_{1,9} = 28.324$ ,  $P < 0.000$ ,  $n = 11$

-S oysters:  $RR \text{ (J h}^{-1}\text{)} = 0.5917 (\pm 0.117) \times AR \text{ (J h}^{-1}\text{)} + 0.028 (\pm 0.017)$ ,  $r^2 = 0.6797$ ,  $F_{1,12} = 25.400$ ,  $P < 0.000$ ,  $n = 14$

Thus, metabolic costs associated with the absorption of 1 Joule energy equivalent — once discounted the standard requirements— would be  $0.179 (\pm 0.033)$  Joules in F oysters, while it would rise to  $0.5917 (\pm 0.117)$  Joules in S oysters. Whilst no differences were found for intercepts, significant differences (Slope comparison test,  $t_{21} = -2.712$ ,  $P < 0.01$ ) were found between slopes. For comparison, this metabolic component associated with food processing and assimilation into tissue growth has been reported to increase from 0.33 in fast- to  $0.56 \text{ J. J}^{-1}$  in slow-growing *C. gigas* (Bayne 1999), and from 0.40 in *S. commercialis* specimens selected for improved growth to  $0.80 \text{ J J}^{-1}$  in un-selected oysters of the same species (Bayne et al. 1999b).

Hedgecock et al. (2007) and Meyer and Manahan (2010) have recently shown that several genes are differentially expressed in fast- versus slow-growing larvae of *C. gigas*. Differences were basically found to affect genes involved in 1) protein metabolism, 2) mitochondrial metabolism and 3) feeding. With regard to feeding, the differences were limited to the small cardio-active peptide precursor gene (*SCPb*) which probably regulates the contractile state of gut muscles. However, the main differences between fast and slow larvae were related to genes involved in protein metabolism:  $\sim 50\%$  of the differential expression corresponded to ribosomal protein genes. Interestingly, slow-growing larvae had less uniform expression of ribosomal protein genes than their fast-growing counterparts. The authors suggested this would lead to deleterious effects on growth and fitness overall.

Since the *C. gigas* larvae in Meyer and Manahan (2010) belonged to the same genetic families used by their co-workers Pace et al. (2006), reported genetic differences involving aspects of protein metabolism can be assumed to underpin physiological traits accounting for inter-individual growth variability. Further analyses are thus needed in order to link differential gene expression among growth categories to phenotypic variation in physiological components of growth.

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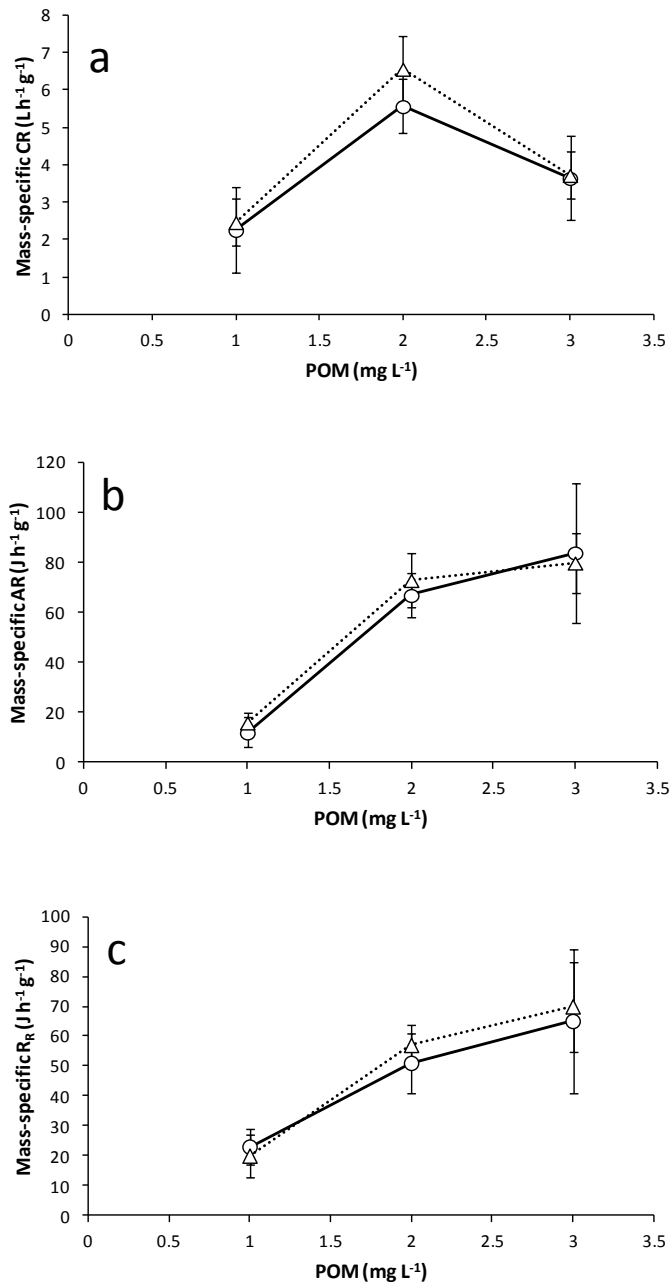
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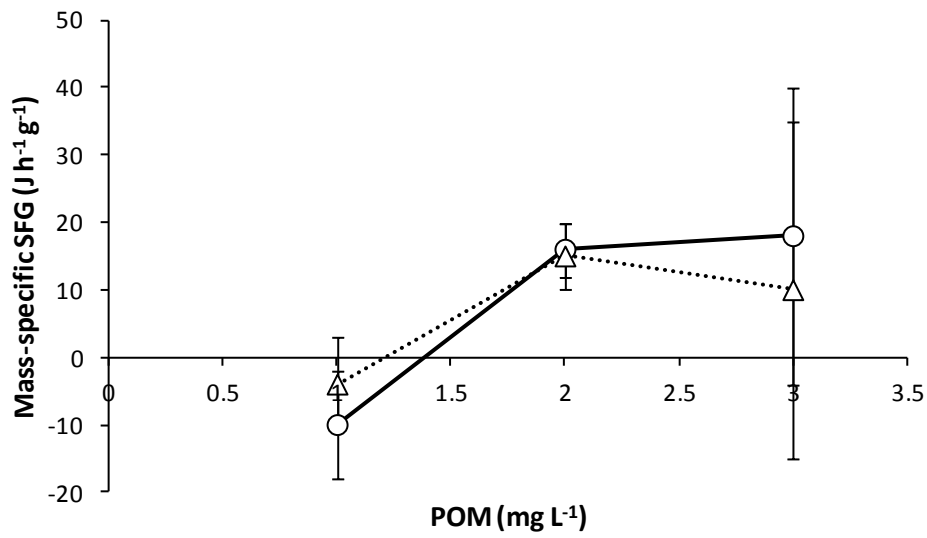
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## Figures

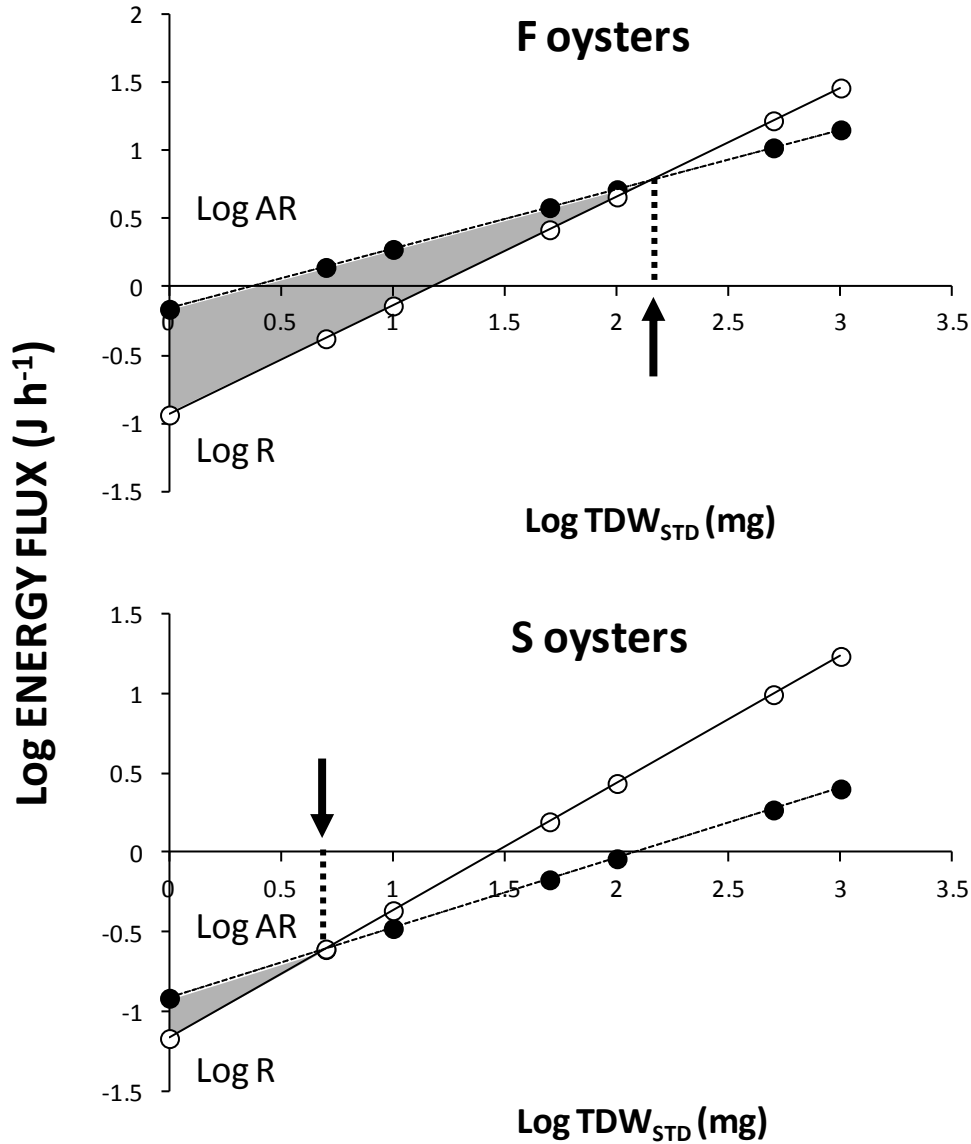


**Fig. 1** Mass-specific values of (a) clearance rate ( $\text{L h}^{-1} \text{g}^{-1}$ ), (b) absorption rate ( $\text{J h}^{-1} \text{g}^{-1}$ ) and (c) routine metabolic rate ( $\text{J h}^{-1} \text{g}^{-1}$ ) in fast (circles) and slow (triangles) growing oysters as a function of particulate organic matter in the diet (POM:  $\text{mg L}^{-1}$ ). Values are mean  $\pm$  SD ( $n=5$ )

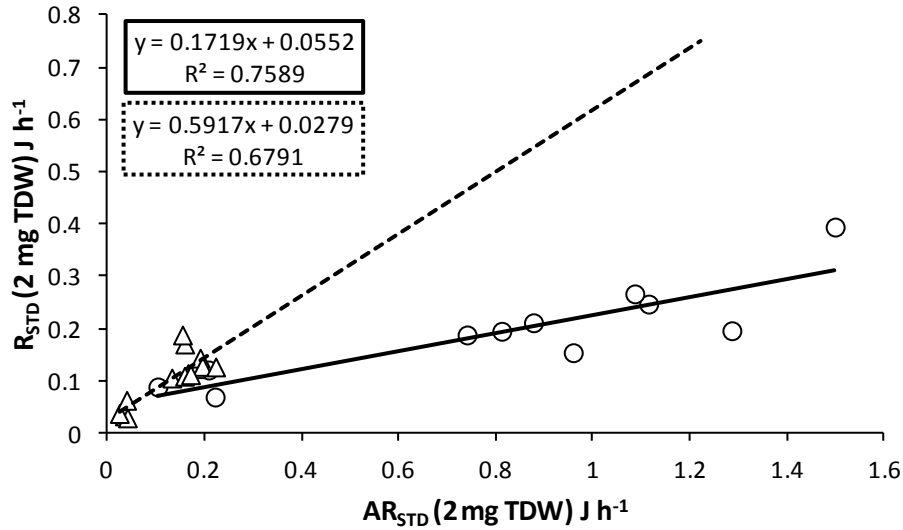


**Fig. 2** Mass-specific scope for growth ( $\text{J h}^{-1} \text{g}^{-1}$ ) in fast (circles) and slow (triangles) growing oysters as a function of particulate organic matter in the diet (POM:  $\text{mg L}^{-1}$ ). Values are mean  $\pm$  SD ( $n=5$ )





**Fig. 3** Log-Log representation of absorption rate (AR) and metabolic rate (R) standardized to a range of sizes (given as mg. TDW<sub>STD</sub>). Experimental rates ( $Y_E$ ) in F (upper figure) and S (lower figure) measured at medium food ration (TPM=  $1.53 \pm 0.18$  mg L<sup>-1</sup>) were standardized using the expression  $Y_{STD} = (\text{TDW}_{STD} / \text{TDW}_E)^b * Y_E$ . Mass-exponents for AR and R were 0.439 and 0.8, respectively (Bougrier et al. 1995). Arrows indicate estimated sizes for SFG = 0 in F and S spat



**Fig. 4** Relationship between standardized (to an equivalent 2 mg of TDW) routine metabolic rate ( $R_{STD}$ :  $J h^{-1}$ ) and absorption rate ( $AR_{STD}$ :  $J h^{-1}$ ) fast- (circles) and slow-growing (triangles) *Crassostrea gigas*. Solid and dashed lines represent performed linear regressions (see text for equations)

## Tables

Ration level	TPM ( $mg L^{-1}$ )	PIM ( $mg L^{-1}$ )	POM ( $mg L^{-1}$ )	OC	Nitrogen content (%)	Carbon content (%)
Low	$0.564 \pm 0.132$	$0.107 \pm 0.034$	$0.456 \pm 0.101$	$0.814 \pm 0.024$	-	-
Medium	$1.531 \pm 0.184$	$0.480 \pm 0.059$	$1.037 \pm 0.111$	$0.684 \pm 0.008$	$10.201 \pm 1.494$	$60.382 \pm 6.888$
High	$2.890 \pm 0.415$	$1.129 \pm 0.280$	$1.760 \pm 0.166$	$0.614 \pm 0.041$	$10.133 \pm 0.376$	$66.285 \pm 4.694$

**Table 1** Characteristics of experimental ration levels used in physiological determinations. TPM: total particulate matter; PIM: particulate inorganic matter, POM: particulate organic matter; OC : organic content, Nitrogen content (%) and Carbon content (%)

**Table 2** Mass-specific physiological variables measured in fast (F) and slow (S) growing oysters fed low, medium and high food rations. CR<sub>SPC</sub>: clearance rate (L h<sup>-1</sup> mg TDW<sup>-1</sup>); OIR<sub>SPC</sub>: organic ingestion rate (mg h<sup>-1</sup> mg TDW<sup>-1</sup>); AE: absorption efficiency; AR<sub>SPC</sub>: absorption rate (mg h<sup>-1</sup> mg TDW<sup>-1</sup>); R<sub>SPC</sub>: respiratory metabolic rate (J h<sup>-1</sup> mg TDW<sup>-1</sup>); SFG<sub>SPC</sub>: scope for growth (J h<sup>-1</sup> mg TDW<sup>-1</sup>). Mean values ± SD (*n* = 5) are presented together with a summary of two-factor ANOVA testing significant effects of growth category and experimental food ration on physiological variables

Mass-specific physiological parameters	Growth group	Low ration (0.5 mm <sup>3</sup> L <sup>-1</sup> )	Medium ration (3.0 mm <sup>3</sup> L <sup>-1</sup> )	High ration (6.0 mm <sup>3</sup> L <sup>-1</sup> )	Summary of two factor ANOVA		
					Growth category	Ration	Interaction
CR <sub>SPC</sub>	F	2.26 ± 1.14	5.57 ± 0.73	3.65 ± 1.13	DF = 1	DF = 2	DF = 2
	S	2.47 ± 0.614	6.57 ± 0.88	3.73 ± 0.62	F = 1.632 p = 0.215	F = 42.123 p < 0.000	F = 0.730 p = 0.494
OIR <sub>SPC</sub>	F	0.001 ± 0.0005	0.0058 ± 0.0007	0.0064 ± 0.0019	DF = 1	DF = 2	DF = 2
	S	0.0011 ± 0.0002	0.0068 ± 0.0009	0.0066 ± 0.0011	F = 1.078 p = 0.311	F = 76.763 p < 0.000	F = 0.564 p = 0.577
AE	F	0.63 ± 0.03	0.62 ± 0.09	0.69 ± 0.04	DF = 1	DF = 2	DF = 2
	S	0.79 ± 0.01	0.57 ± 0.03	0.65 ± 0.04	F = 1.497 p = 0.234	F = 13.066 p < 0.000	F = 12.322 p < 0.000
AR <sub>SPC</sub>	F	0.012 ± 0.006	0.067 ± 0.009	0.084 ± 0.028	DF = 1	DF = 2	DF = 2
	S	0.016 ± 0.004	0.073 ± 0.011	0.08 ± 0.012	F = 0.112 p = 0.741	F = 60.213 p < 0.000	F = 0.344 p = 0.713
R <sub>SPC</sub>	F	0.023 ± 0.006	0.051 ± 0.01	0.065 ± 0.024	DF = 1	DF = 2	DF = 2
	S	0.02 ± 0.007	0.057 ± 0.007	0.0699 ± 0.015	F = 0.174 p = 0.681	F = 25.758 p < 0.000	F = 0.340 p = 0.716
SFG <sub>SPC</sub>	F	-0.01 ± 0.008	0.016 ± 0.004	0.018 ± 0.022	DF = 1	DF = 2	DF = 2
	S	-0.004 ± 0.007	0.015 ± 0.005	0.01 ± 0.025	F = 0.049 p = 0.827	F = 5.286 p = 0.015	F = 0.397 p = 0.678

**Table 3** C:N ratios and elemental absorption efficiencies of medium and high ration diets in fast (F) and slow (S) growing oysters.  $AE_C$ : Carbon absorption efficiency;  $AE_N$ : Nitrogen absorption efficiency. Values are mean  $\pm$  SD ( $n = 5$ )

Parameter	Growth group	Medium ration (3.0 mm <sup>3</sup> L <sup>-1</sup> )	High ration (6.0 mm <sup>3</sup> L <sup>-1</sup> )
C:N diet		5.94 $\pm$ 0.19	6.56 $\pm$ 0.16
C:N faeces	F	6.56 $\pm$ 0.35	7.27 $\pm$ 0.38
	S	6.72 $\pm$ 0.18	6.96 $\pm$ 0.18
$AE_C$	F	0.697 $\pm$ 0.090	0.775 $\pm$ 0.024
	S	0.647 $\pm$ 0.010	0.724 $\pm$ 0.060
$AE_N$	F	0.723 $\pm$ 0.093	0.797 $\pm$ 0.029
	S	0.689 $\pm$ 0.010	0.741 $\pm$ 0.054

**Table 4** Summary of the two factor ANOVA testing significant differences between growth categories (F vs S) and experimental rations for C:N ratios in faeces, carbon absorption efficiency (AE<sub>C</sub>) and nitrogen absorption efficiency (AE<sub>N</sub>)

<i>Parameter</i>	<i>Source of variation</i>	<i>DF</i>	<i>MS</i>	<i>SS</i>	<i>F</i>	<i>P</i>
C:N ratios (faeces)	G.category	1	0.025	0.025	0.320	0.580
	Ration	1	0.979	0.979	12.358	0.003
	Interaction	1	0.241	0.241	3.046	0.103
	Error	14	0.079	1.109		
AE <sub>C</sub>	G.category	1	0.012	0.012	3.892	0.069
	Ration	1	0.027	0.027	9.078	0.009
	Interaction	1	1.859 x 10 <sup>-6</sup>	1.859 x 10 <sup>-6</sup>	0.001	0.980
	Error	14	0.003	0.042		
AE <sub>N</sub>	G.category	1	0.009	0.009	3.133	0.099
	Ration	1	0.018	0.018	6.003	0.028
	Interaction	1	0.001	0.001	0.175	0.682
	Error	14	0.003	0.041		