Marine Environmental Research 180 (2022) 105725

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Differential tissue development compromising the growth rate and physiological performances of mussel

Maitane Pérez-Cebrecos [a,b,*], Daniel Prieto [a], Esther Blanco-Rayón [b,c], Urtzi Izagirre [b,c], Irrintzi Ibarrola [a]

[a] Department of Genetics, Physical Anthropology and Animal Physiology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain
[b] Research Centre for Experimental Marine Biology and Biotechnology (PiE – UPV/EHU), University of the Basque Country (UPV/EHU), Plentzia, Spain
[c] CBET Research Group, Department of Zoology and Animal Cell Biology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain

A R T I C L E I N F O

Keywords:
Mytilus galloprovincialis
Growth rate
Energetic metabolism
Tissue analysis
Digestive gland atrophy
Gill
Antioxidant enzymes
Biochemistry

A B S T R A C T

Differences in the food acquisition rates and in the energetic costs of metabolism seem to affect the growth rate variability of mussels. The aim of this study was to analyze if the physiological performances responsible for such growth rate variability are accompanied by structural differences at tissue or cellular level in the main organs involved in energy acquisition (gill) and processing (digestive gland). Fast growers had higher cilia density and metabolic efficiency in their gill, and well-developed digestive tissue with barely no connective tissue or atrophy. Slow-growing mussels displayed stress signs that impede the proper acquisition, digestion and absorption of food: low cilia density, low mitochondrial capacity and high antioxidant activity levels in the gills, and high atrophy of the digestive gland. The data herein explains the growth rate variability of mussels, demonstrating that morphological and functional differences exist between fast and slow growers.

1. Introduction

Survival, maturity, reproductive success and other vital rates are frequently related to growth and body size (Peters, 1983), turning growth into one of the most important but variable biological traits. In bivalves, growth rates vary extraordinarily not only under laboratory conditions (e.g., Brown, 1988), but also in nature (e.g., Brown, 1988; Gaffney and Scott, 1984; Mallet and Haley, 1983), and even for sibling specimens (e.g., Tamayo et al., 2014). Understanding how growth variability works in mussels would help to analyze the dynamics of the population (Fuentes-Santos et al., 2018; Vincenzi et al., 2014). In addition, improving production through the selection of growth lines helps develop efficient, sustainable and predictable aquaculture systems (Gu et al., 2011; Li et al., 2011; Zhang et al., 2018, 2021).

It has been recently proposed (Fuentes-Santos et al., 2018) that differences in the physiological patterns may be direct drivers for growth variability, not just a consequence of different growing capacities. Most of the studies measuring the physiological performance of even-aged individuals have been performed after the size differentiation into fast (F) and slow (S) growers has occurred. The physiological performance has been studied in oysters (Pernet et al., 2008; Tamayo et al., 2014), clams (Tamayo et al., 2011, 2013, 2015), and mussels (Fernández-Reiriz et al., 2016; Prieto et al., 2018, 2020). These studies established a connection between inter-individual growth dissimilarities and the food-acquisition rates that might be coupled with differences in the energetic costs associated with metabolism depending on the feeding conditions. However, a whole characterization at other biological organization levels would be needed to know if structural differences accompany the differing physiological performances.

Out of the physiological components of food acquisition, the clearance rate (CR) is the most influential one. The CR is carried out by the gills and to analyze the potential role of this organ as the primary determinant for differential growth, the gill-surface area has been studied and shown to be greater per unit mass in F mussels (Prieto et al., 2018, 2019; Tamayo et al., 2011). Since the effective filter in the food acquisition process is the ciliary arrangement of the gill filaments (Morton, 1983), there is a need to deeply assess, together with the gill area, the status of the cilia in the different growing groups.

Cilia arrangement and building depend mainly upon the microtubule network, which is known to be disrupted or destabilized under oxidative

* Corresponding author. Department of Genetics, Physical Anthropology and Animal Physiology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain.
E-mail address: maitane.perez@ehu.eus (M. Pérez-Cebrecos).

https://doi.org/10.1016/j.marenvres.2022.105725
Received 8 April 2022; Received in revised form 27 July 2022; Accepted 4 August 2022
Available online 15 August 2022
0141-1136/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

stress (Kratzer et al., 2012; Tamura et al., 2020). Thus, it would be important to analyze the implication on growing patterns and cilia structure that oxidative stress can have. Catalase (CAT) is a traditionally measured antioxidant enzyme involved in the initial anti-oxidative mechanism and is widely used as a biomarker of oxidative stress (e.g., Pampanin et al., 2005; Romèo et al., 2003). Glutathione S-transferase (GST) catalyzes the conjugation of reduced glutathione to cellular components damaged by reactive oxygen species (ROS), leading to their detoxification (Storey, 1996), thus playing a major role in antioxidant defense and being useable as an oxidative stress biomarker (e.g., Hellou et al., 2012). In this framework, the role of mitochondria in relation to the energy demand that cilia ask for would be another target worthy of testing amongst differently growing mussels. The activity of cytochrome c oxidase (COX) has been used lately to measure mitochondrial capacity (Sokolova, 2018), and it has been recently adapted to be determined in mussels (Blanco-Rayón et al., 2019[a]).

In the context of inter-individual growth differences, the digestive gland has received little attention, particularly in mussels, despite it functions primarily as the organ of intracellular digestion and absorption (Morton, 1983). It is also the storage site for metabolic reserves and a participant in the transference of metabolic reserves to other organs (Cartier et al., 2004). Moreover, its morphological appearance has been proved to be of great plasticity under different food availability scenarios (e.g., Morton, 1983; Robinson et al., 1981, 1983). In fact, it modulates the size of the digestive diverticula and their cellular volume (Ibarrola et al., 2000). However, there is no available data on whether this type of adjustments happen along with food acquisition capacity or different growth rates. These studies provided the basic knowledge to understand the digestive regulation of bivalves but must be completed with morphological assessments that uphold the physiological performances of differentially growing individuals.

Besides the morphofunctional analysis of the gill and the digestive gland as keys to understanding fast and slow growth, the response to different nutritive conditions would provide valuable information about their plasticity. Previous studies have reported differences between fast and slow growing mussels exposed to different diets (Prieto et al., 2020), where different physiological profiles were recorded irrespective of the ration. However, if a better understanding of the growing patterns is pursued, research should be done on the implications of those physiological differences at other biological organization levels.

As stated above, variation in the growth of mussels has implications for both individual fitness and population dynamics. Comprehending such variation would be valuable for conservation and management and, therefore, would help improve aquaculture production (Kendall and Fox, 2002). Here we tackle the individual variation in the growth of mussels from a multidisciplinary approach to obtain a holistic understanding of the organization and function of the growing patterns. The work aims to establish a connection between the physiological performances of the different growing profiles, and the tissue structure and cellular activity of the two main organs involved in energy acquisition and processing.

2. Materials and methods

2.1. Collection of mussel seeds and experimental setup

Mussel seeds, *Mytilus galloprovincialis*, were collected in February 2020 from monolayer mussel beds growing in a rocky intertidal area located in Ibarrangelu (Biscay, Spain, 43°24' N; 2°40' W). Mussels were transferred to the laboratory in air-exposed wet containers at ambient temperature.

At the laboratory, the shell length of all the individuals was measured with electronic calipers and 300 homogeneously sized individuals (shell length of 10.98 ± 0.52 mm) were sorted for experiments and placed in a tank (50 L) at constant seawater salinity (33PSU) and temperature (18 °C). The selected seeds were reared in the laboratory during a 3-month period under a constant food supply that consisted of a suspension of cultured algae *Isochrysis galbana* (T-Iso) constantly dosed at 20000 cells · mL^{-1}. The concentration was maintained stable by frequently checking with a Coulter Multisizer 3 and homogeneity ensured with air circulation. During the rearing period, the tanks were cleaned up and seawater-renewed twice a week. When cleaned, mussels were separated from one another by gently cutting the byssus to avoid inter-individual competition for food.

The first week of the rearing period, the clearance rate and the oxygen consumption was determined in 20 randomly selected individuals. Those same individuals were then dissected for histological analysis. The remaining set of mussel seeds was maintained for three months. After this period, the largest 40 (fast-growers – F), the 40 medium-sized mussels (intermediate growers – I) and the smallest 40 (slow-growers – S) were selected to be acclimatized under two different food-rations for two weeks. Half of the mussels (n = 20 for each growing condition) were fed with a high ration (50000 cells · mL^{-1}) and the other half with a low ration (10000 cells · mL^{-1}).

After the acclimation period, 10 individuals from each group above were used to measure the main components of the energy balance (clearance rate –CR– and routine metabolic rate –RMR–). Once the physiological measurements were completed, whole animal flesh (for the smallest individuals) and a cross section including mantle, gills and digestive gland (for the medium-size and largest mussels) were extracted for histological examination. The gills of 10 additional mussels from each of those six groups were frozen in liquid nitrogen and stored at -80 °C for biochemical analysis.

2.2. Biometry

The shell-lengths and live weights of individual mussels were measured once every two weeks using 0.05 mm accuracy calipers and a 0.01 mg accuracy balance.

2.3. Physiological parameters

2.3.1. Clearance rate (CR: $L \cdot h^{-1}$)

Clearance rate was measured by placing the mussels in experimental glass bottles of 250 mL with rounded edges, the flow rates of which were adjusted to obtain a reduction of 15–30% on the particle concentration compared with the control chamber. Samples of water in the outflow of individual and control chambers were taken every hour during 11–12 h. Thus, the CR of each individual was calculated as the mean value of 11–12 determinations during the whole day, and according to the expression proposed by Hildreth and Crisp (1976) :

$$CR = F \cdot ((C_i - C_0) / C_i),$$

where F is the flow rate ($L \cdot h^{-1}$), C_i is the particle concentration in the control outflow and C_0 the particle concentration in the outflow of the experimental chamber. Particle concentrations were determined with a Counter Coulter Z1.

2.3.2. Metabolic expenditure (RMR: $mL\ O_2 \cdot h^{-1}$)

Routine metabolic rate was assessed by measuring oxygen consumption. Mussels were removed from feeding chambers and introduced into respirometers of around 50 mL sealed with LDO oxygen probes connected to oximeters (HATCH HQ40d). Rates of oxygen consumption were computed from the decline in oxygen concentration in the chambers registered during 3–4 h, or until values decreased 20–30% of initial baseline, every 5–10 min. A control chamber was used to check the stability of the oxygen concentration.

2.3.3. Size standardization of physiological rates

CR and RMR were standardized to a common live weight of 1 g, according to the expression:

$$Y_{STD} = (1/W_{EXP})^b \cdot Y_{EXP},$$

in which Y_{STD} and Y_{EXP} represent, respectively, standard and experimental physiological rates and W_{EXP} represents the experimental live weight of the individual. The power values used to scale physiological rates to body weight (b) were 0.58 (Bayne and Hawkins, 1997) for CR and 0.724 (Bayne et al., 1973) for oxygen consumption.

2.4. Gill surface area (GA: $mm^2 \cdot g^{-1}$)

Photographs of the gill of 20 mussels were taken with a digital camera, and the surface area of the gills from each individual was calculated using ImageJ software (National Institutes of Health). As a means to ensure correct sizing, millimetric paper was placed under the mussel when taking the photograph. Data shown correspond to one side of the demibranch. Gill areas were standardized for an equivalent of 1 g live-weight mussel according to the formula:

$$GA_{STD} = (1/W_{EXP})^b \cdot GA_{EXP},$$

where GA_{STD} and GA_{EXP} represent the standardized and experimental gill areas, respectively, and W_{EXP} is the experimental live weight of the mussel. The power function used to scale gill area to live weight was 0.66 (Vahl, 1973; Hawkins and Bayne, 1992; Jones et al., 1992).

2.5. Histology

The whole organisms or cross sections (n = 10 per experimental group) were fixed in seawater with 4% formaldehyde, dehydrated in an ethanol bath series, paraffin-embedded using a Leica ASP3005 tissue processor and sectioned at 5 μm with a Leica RM2125RTS microtome. Paraffin sections were stained with two different staining procedures. On the one hand, hematoxylin-eosin (H/E) staining was used to analyze the digestive gland, gills and mantle. On the other hand, toluidine-eosin staining was employed to better discriminate basophilic cells in the digestive gland (Blanco-Rayón et al., 2019[b]).

2.5.1. Digestive gland assessment

The digestive tissue ratio (CTD), the changes in volume density of basophilic cells (Vv_{BAS}) and the atrophy of the epithelium of the digestive alveoli were measured.

CTD and Vv_{BAS} were quantified through stereology, by counting three randomly selected fields in each slide at 40× objective (final magnification ~400×) and employing a drawing tube attached to a light microscope. A simplified version of the Weibel graticule multipurpose test system M-168 (Weibel, 1979) was used to record the hits on basophilic cells (b), digestive cells (d), diverticular lumens (l) and interstitial connective tissue (c). CTD ratio was calculated as CTD = c/(b + d + l). Vv_{BAS} was reckoned following Delesse's principle (Weibel, 1979), as V_{BAS}/VEP, where V_{BAS} is the volume of basophilic cells and VEP the volume of digestive gland epithelium.

Following Kim et al. (2006), a grading from 0 to 4 was used to calculate the atrophy index of the digestive alveoli. In that classification, 0 means normal digestive diverticula with nearly occluded lumen; 1 means co-occurrence of normal and partially atrophied tubules of epithelium thickness greater than one-half of normal; 2 means digestive epithelium thickness half of normal; 3 means significantly atrophied tubules with digestive epithelium less than half as thick as normal, and 4 means that digestive epithelium is extremely thin and nearly all tubules are affected.

2.5.2. Adipogranular cell density

The adipogranular (ADG) cell density was estimated as described by Bignell et al. (2008) using a grading system, where 0 means no ADG cells apparent within vesicular connective tissue, 1 means ADG cells can be seen but they appear to be scarce, 2 means ADG cells appear scattered throughout mantle tissue, 3 means there is a marked increase in the abundance of ADG cells and some areas may not appear to show absolute consistency, and 4 means ADG cells can be seen to constitute the majority of connective tissue volume.

2.5.3. Gill structure

To assess the gill structure a grading system was designed based on the frontal and latero-frontal cilia density and epithelium organization (Table 1). Normal cilia density in most lamella and well-organized epithelium was graded with the highest score. When average cilia density was less than normal in most lamella, but epithelium was still well organized, the scoring lowered to one. The scoring was the lowest when average cilia density was less than normal in nearly all lamella and the epithelium showed an evident disorganization.

2.6. Biochemical determinations in the gill

Gills of 10 mussels from each of the six experimental groups were individually homogenized in 0.05 M potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at 10.000 g for 20 min at 4 °C and the supernatants were stored at - 80 °C until analysis. The gill samples were analyzed for glutathione S-transferase (GST), catalase (CAT) and cytochrome c oxidase (COX) enzymes. All enzyme activities were measured in 96-well plates using a microplate reader (TECAN Infinite 200), analyzed using Magellan software (TECAN) and were expressed as a function of the protein concentration in the samples. The protein concentration was determined in triplicate according to Bradford's method adapted to a microplate and using γ-bovine globulins as standard (Guilhermino et al., 1996).

CAT activity was measured as degradation of hydrogen peroxide (H2O2, Fluka 95302) mediated by CAT at 240 nm (Claiborne, 1985). GST activity was measured as the formation rate of the conjugated substrate chlorodinitrobenzene-glutathione (CDNB-GSH) at 340 nm, according to Habig et al. (1974). When measuring COX activity, in brief, isolation and assay conditions were as follows: homogenization buffer: 25 mM potassium phosphate, pH 7.2, 10 μg/ml PMSF, 2 μg/ml aprotinin; assay: 20 mM potassium phosphate, pH 7.0, 16 μM reduced cytochrome c(II), 0.45 mM n-dodecyl-b-d-maltoside, 2 μg/ml antimycin A; acquisition wavelength: 550 nm.

2.7. Statistical analysis

Data was evaluated first for normality and homoscedasticity by means of Shapiro-Wilk and Levene's test, respectively. In those cases where normality was not followed, data were logarithmically transformed after which normality was held. Significant effects exerted by growth-condition (F, I or S) and food ration (high or low) on physiological and histological measurements were analyzed employing a two-way ANOVA. As a post hoc Tukey (homogeneity of variances) or Games-Howell (no homogeneity of variances) tests were applied. Semi-quantitatively gathered data was analyzed through non-parametric tests. For Kruskal-Wallis, Dunn's test was applied as post hoc. Statistical analyses were performed using IBM SPSS Statistics 25 (IBM Corp. Released, 2017). Covariance analysis (ANCOVA; Zar, 2010) was used to test the significance of differences between regression coefficients for

Table 1
Semi-quantitative scale for gill structure assessment.

Score	Description
0	Frontal and latero-frontal cilia density is low, and cell damage and a disorganized epithelium is evident
1	Frontal and latero-frontal cilia density is low but the epithelium is well organized
2	Frontal and latero-frontal cilia density is high and the epithelium is well organized

the different growth rates.

3. Results

3.1. Growth rate of fast and slow growers

Inter-individual differences in the growth rates were evident enough after 3 months as to easily select fast, intermediate and slow growers. F individuals were 3.5 times heavier than S individuals were, and their shell length was almost 70% larger than S mussels shells (Fig. 1). Indeed, if the growth rates (mm/day) of the selected mussels are computed by adjusting linear regression models to the variations of the mean values of shell lengths with time, the following equations arise:

$$\text{Fast growers: } 0.163\ (\pm 0.002) \times time\ (days) + 10.979\ (\pm 0.057),\quad F = 8899.1,\ p < 0.0001$$

$$\text{Intermediate growers: } 0.110\ (\pm 0.001) \times time\ (days) + 10.979\ (\pm 0.036),\quad F = 9218.2,\ p < 0.0001$$

$$\text{Slow growers: } 0.052\ (\pm 0.001) \times time\ (days) + 10.979\ (\pm 0.038),\ F = 1817.9,\ p < 0.0001$$

Under maintenance conditions, fast growers grew an average of 0.163 mm/day, intermediate growers 0.110 mm/day and slow growers 0.052 mm/day. Analysis of covariance revealed significant differences for the slopes (slope test: F value = 1066.038, df = 1026, $p < 0.05$), and multiple comparison among slopes revealed that the three of them were statistically different from one another (bF vs. bS: q = 79.972, $p < 0.05$; bF vs. bI: q = 38.619, $p < 0.05$; bS vs. bI: q = 40.678, $p < 0.05$).

3.2. Physiological components of the energy balance

3.2.1. Clearance rate (CR: $L \cdot h^{-1} \cdot g^{-1}$)

Fast-growing individuals attained significantly 2 times higher CR values than their slow-growing counterparts did (0.429 ± 0.19 L $h^{-1} \cdot g^{-1}$ vs. 0.216 ± 0.12 L $h^{-1} \cdot g^{-1}$, respectively), while I individuals displayed intermediate values (0.321 ± 0.15 L $h^{-1} \cdot g^{-1}$) not different to those of F and S mussels (Fig. 2). For both food rations, the three growth-condition groups followed a quite similar pattern. CR values were significantly 2 times higher in the mussels fed with the low-concentrated ration (0.411 ± 0.16 L $h^{-1} \cdot g^{-1}$) when compared to the mussels fed the high food ration (0.233 ± 0.15 L $h^{-1} \cdot g^{-1}$). Accordingly, the two-way

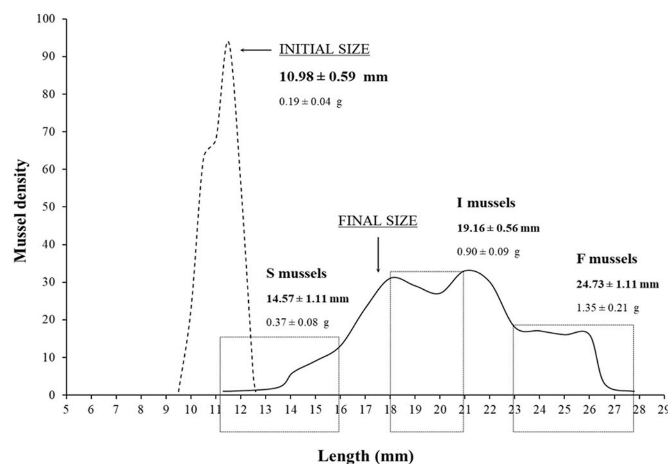
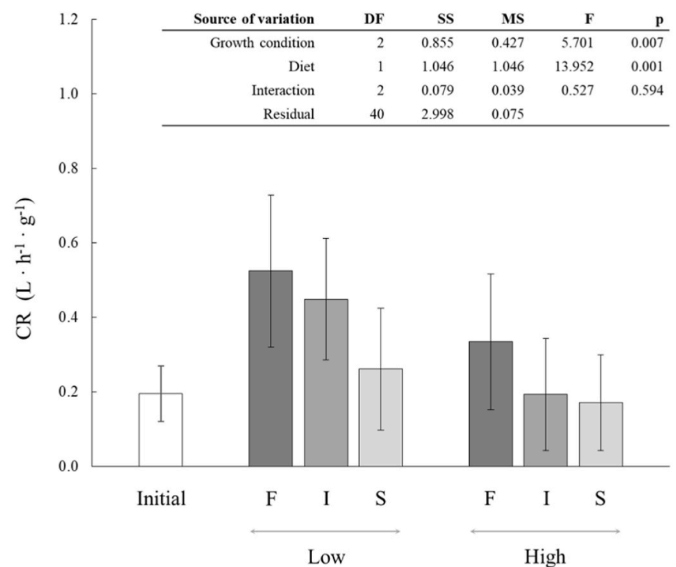


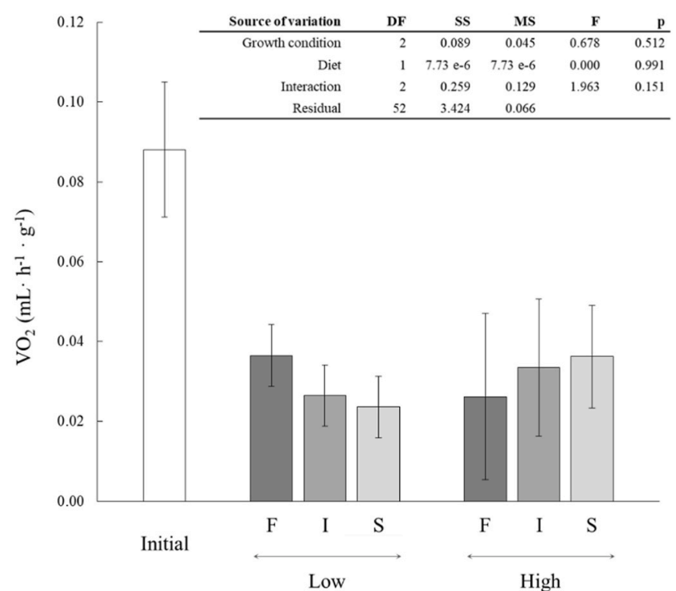
Fig. 1. Shell-length distribution of the mussels upon arrival to the laboratory (initial size) and after three months (final size). The boxes delimit the size range of the selected F, I and S mussels; their corresponding shell-lengths (mm) and live weights (g) (mean ± standard deviation) are indicated.



Source of variation	DF	SS	MS	F	p
Growth condition	2	0.855	0.427	5.701	0.007
Diet	1	1.046	1.046	13.952	0.001
Interaction	2	0.079	0.039	0.527	0.594
Residual	40	2.998	0.075		

Fig. 2. Clearance rate ($L \cdot h^{-1} \cdot g^{-1}$) of fast (F), intermediate (I) and slow (S) growing mussels for low-concentrated and high-concentrated rations. The clearance rate from the initial determination is also depicted. Intervals indicate standard deviation. On the top, the two-way factor ANOVA testing significant effects of growth condition (F, I or S) and ration (Low or High) is shown.

ANOVA showed in Fig. 2 indicates that both growth condition and ration, but not the interaction, exerted a significant effect on the CR of the mussels. The mean CR of mussels recorded during the first week of the experiment is shown in the figure for comparative purposes. Although no attempt of statistical testing has been made, the maintenance of mussels in the laboratory under the condition of continuous feeding exerted a positive effect upon the CR in F an I mussels but not S mussels.



Source of variation	DF	SS	MS	F	p
Growth condition	2	0.089	0.045	0.678	0.512
Diet	1	7.73 e-6	7.73 e-6	0.000	0.991
Interaction	2	0.259	0.129	1.963	0.151
Residual	52	3.424	0.066		

Fig. 3. Oxygen consumption ($mL \cdot h^{-1} \cdot g^{-1}$) of fast (F), medium (I) and slow (S) growing mussels for low-concentrated and high-concentrated rations. The oxygen consumption from the initial determination is also depicted. Intervals indicate standard deviation. On the top, the two-way factor ANOVA testing significant effects of growth condition (F, I or S) and ration (Low or High) is shown.

3.2.2. Routine metabolic rate (RMR: mL $O_2 \cdot h^{-1} \cdot g^{-1}$)

Mean values of routine metabolic rate are plotted in Fig. 3. The oxygen consumption of mussels was found to decrease sharply during the rearing period. The mean RMR value was 0.09 ± 0.017 mL $O_2 \cdot h^{-1} \cdot g^{-1}$ during the first week at the laboratory and trebled that of the mean oxygen consumption recorded for selected F, I and S mussels: 0.03 ± 0.005 mL $O_2 \cdot h^{-1} \cdot g^{-1}$. The two-factor ANOVA indicates that neither the growth condition nor the food ration factors exerted any significant effect on the RMR.

3.3. Gill-surface area

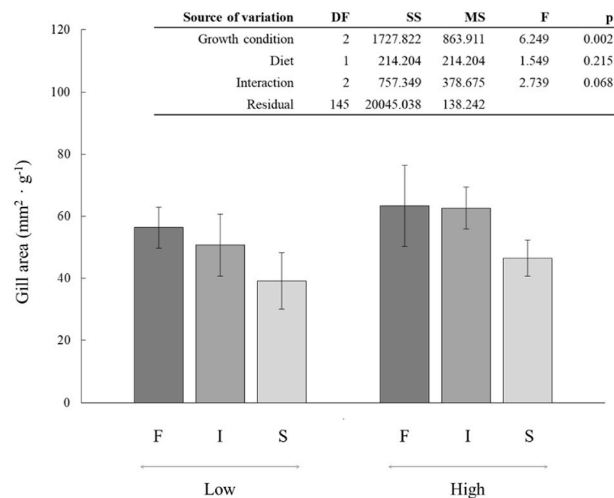
Mean values (±SD) of the gill-surface area and the two-way factor ANOVA are shown in Fig. 4. The two-way ANOVA indicates that irrespective of the ration, there are differences among the gill-surface area values of the growing groups. Tukey test revealed that F mussels had significantly 40% larger gill-surface area than S mussels ($p < 0.001$): average value of F growers is around 70 mm^2 g^{-1}, whereas that of S mussels about 40 mm^2 g^{-1}. The value of intermediate growers falls down to a mid-value of around 56 mm^2 g^{-1}, which is significantly different from the value of S mussels ($p < 0.001$), but not from that of F mussels ($p = 0.077$), according to post hoc tests.

3.4. Histological analysis of the digestive gland

The results of the stereological analysis of the digestive gland of F, I and S mussels fed low and high food concentrations are compiled in Table 2, together with a summary of the two-factor analysis of variance. The initial values of each parameter are also shown.

3.4.1. Connective-to-digestive (CTD) ratio of the digestive gland

Growth condition, food ration and the interaction term exerted significant effects on the CTD ratio. Mean CTD in S mussels (0.518 ± 0.44) was almost 3 times higher than that in fast (0.168 ± 0.11) and intermediate (0.232 ± 0.14) growers (Fig. 5: A,B), being that difference significant. The mussels fed low concentrated ration attained approximately 1.5 times higher CTD values (0.382 ± 0.31) than mussels fed high food ration (0.230 ± 0.15). However, ration affected the CTD values of both I and S mussels (Table 3) but not F mussels, thus resulting in a significant interaction.



Source of variation	DF	SS	MS	F	p
Growth condition	2	1727.822	863.911	6.249	0.002
Diet	1	214.204	214.204	1.549	0.215
Interaction	2	757.349	378.675	2.739	0.068
Residual	145	20045.038	138.242		

Fig. 4. Gill area ($mm^2 \cdot g^{-1}$) of fast (F), intermediate (I) and slow (S) growing mussels for low-concentrated and high-concentrated rations. Intervals indicate standard deviation. On the top, the two-way ANOVA testing significant effects of growth condition (F, I or S) and ration (Low or High) is shown.

3.4.2. Volume density of basophilic cells (Vv_{BAS})

Growth condition significantly affected VvBAS: post hoc Tukey test showed that S individuals had significantly lower values than F and I growers (S vs. F: $p = 0.000$; S vs. I: $p = 0.000$), being this last two statistically equal to each other (F vs. I: $p = 0.089$). Although there is an upward trend of the mean VvBAS value as the food ration decreases, the differences between rations did not achieve the significance ($p = 0.057$). The interaction term was found to be significant.

3.4.3. Atrophy index of digestive alveoli

The mean values for the atrophy index of the digestive alveoli are presented in Fig. 6 (A), along with a summary of the K–W test. The test indicates the existence of significant differences in the

Atrophy index between mussels of different growth-condition: slow-growing mussels had significantly fewer adipogranular cells than fast and intermediate growers did (Dunn's test results: S vs. F: $p = 0.000$; S vs. I: $p = 0.016$; F vs. I: $p = 0.176$) (Fig. 5: C,D). No effect was exerted by the ration factor.

3.5. Histological analysis of the gill

In Table 3, the mean values for the semi-quantitative analysis of the gill appearance are shown. Growth category exerted a significant effect on gill structure index, where slow growers had a lower frontal and latero-frontal cilia density and a disorganized structure of the epithelium when compared to fast and intermediate growers (Dunn's test results: S vs. F: $p = 0.000$; S vs. I: $p = 0.004$; F vs. I: $p = 0.325$) (Fig. 5: E,F). No significant differences were found between the gill appearances of mussels fed under different rations. Moreover, the initial value obtained is closer to that of F and I mussels than to that of S individuals.

3.6. Adipogranular cell index

The mean values for the adipogranular cell index are presented in Fig. 6 (B), along with a summary of the K–W test. The test indicates the existence of significant differences in the adipogranular cell density between mussels of different growth-condition: slow-growing mussels had significantly fewer adipogranular cells than fast and intermediate growers did (Dunn's test results: S vs. F: $p = 0.000$; S vs. I: $p = 0.016$; F vs. I: $p = 0.176$) (Fig. 5: G,H). Again, no effect was exerted by the ration factor. No values for the initial adipogranular cell index are available due to a lack of this type of energy-storage tissue in the samples, virtually all individuals showing well-developed gonadal tissue.

3.7. Biochemical measurements in the gill

Catalase activity (μmol·min-1·mg protein-1) in the gills of mussels showed statistical differences for both ration and growth condition factors (Table 4). Mussels fed low concentration diet had a higher catalase activity than those fed a high concentration diet (18371.55 ± 4227.04 vs. 11187.75 ± 5279.62). On the other hand, post hoc tests showed that the catalase activity of slow growers (21569.78 ± 3725.64) significantly doubled that of fast growers (8927.75 ± 3696.45), and both ends were significantly different from the activity shown by the intermediate growers (14381.42 ± 6837.89) (S vs. F: $p = 0.000$; S vs. I: $p = 0.000$; F vs. I: $p = 0.008$). It should be noted that the interaction between the tested factors was also significant. GST activity (nmol·min-1·mg protein-1) showed no statistical differences between the two rations (Table 4). However, the values obtained for slow growers (10.11 ± 4.85) were significantly higher than those of fast (2.67 ± 1.51) and intermediate growers (3.25 ± 2.89) (S vs. F: $p = 0.000$; S vs. I: $p = 0.000$; F vs. I: $p = 0.840$). COX activity (nmol·min-1·mg protein-1) was also affected by both factors, where mussels at high concentrated ration had higher activity than those at low concentrated ration (220.39 ± 34.17 vs. 185.12 ± 32.36), and fast growers higher than the other two growth-condition groups (S vs. F: $p = 0.001$; S vs. I: $p = 0.328$; F vs. I: $p = 0.044$)

Table 2
Tissue-level biomarkers measured in fast (F), medium (I) and slow (S) growing mussels fed low and high concentrated food rations. CTD ratio: Connective to Digestive ratio; Vv_{BAS}: basophilic cell volume density. Mean values (±SD) are presented together with a summary of two-factor ANOVA testing significant effects of growth condition and experimental food-ration. N = 10 each experimental group. Initial values are shown on the left.

Tissue-level biomarkers	Initial	Growth group	Low concentration (10000 cells·mL^{-1})	High concentration (50000 cells·mL^{-1})	Source of interaction		
			Mean (±SD)	Mean (±SD)	Growth condition	Ration	Interaction
CTD ratio	0.071 (±0.07)	F	0.163 (±0.12)	0.173 (±0.11)	DF = 2	DF = 1	DF = 2
		I	0.344 (±0.21)	0.119 (±0.08)	F = 34.41	F = 11.34	F = 8.48
		S	0.638 (±0.61)	0.399 (±0.26)	p = 0.000	p = 0.001	p = 0.000
Vv_{BAS}	0.213 (±0.06)	F	0.351 (±0.07)	0.241 (±0.06)	DF = 2	DF = 1	DF = 2
		I	0.232 (±0.07)	0.288 (±0.07)	F = 19.79	F = 3.67	F = 13.72
		S	0.401 (±0.12)	0.366 (±0.15)	p = 0.000	p = 0.057	p = 0.000

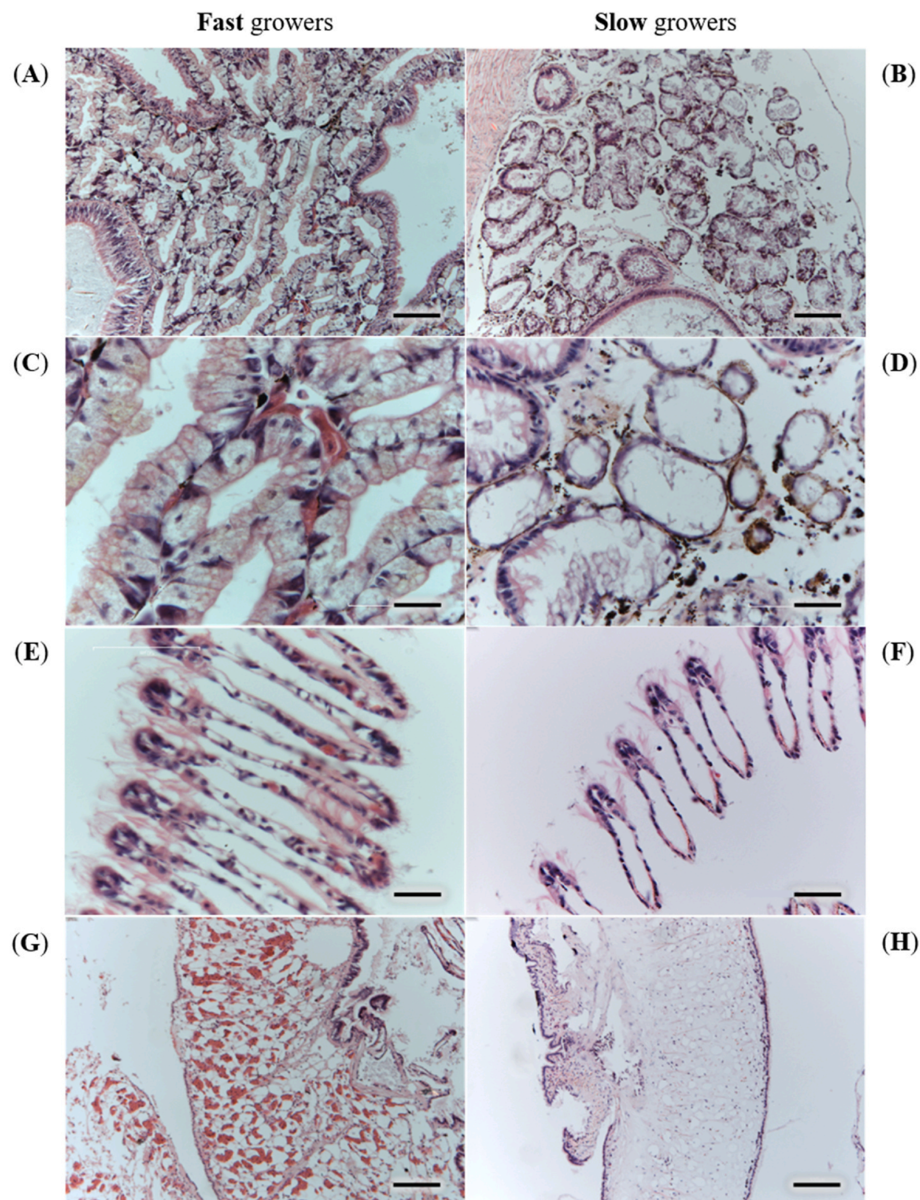
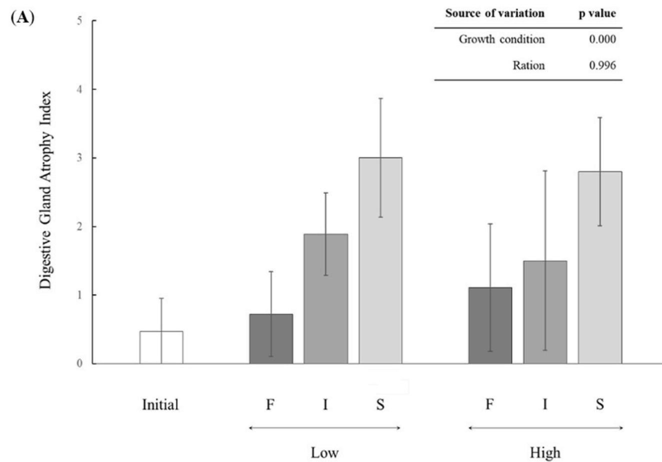


Fig. 5. H-E stained micrographs of fast (a, c, e and g) and slow-growing (b, d, f and h) Mytilus galloprovincialis. (A) and (B) digestive gland tissue; (C) and (D) details at higher magnification of the tissue; (E) and (F) gill tissue; (G) and (H) mantle tissue with. Scale bars: (A), (B), (G) and (H) 100 μm; (C), (D), (E) and (F) 25 μm.

Table 3
Mean - values (±SD) of the semi-quantitative scoring recorded for the gill appearance in the three growing groups (F, I and S) under both food rations. On the left, the initial value. On the right a summary of the Kruskal-Wallis testing the effects of growth condition and experimental diet is shown.

Initial value	Growth condition	Low concentration (10000 cells·mL^{-1})	High concentration (50000 cells·mL^{-1})	Summary of Kruskal-Wallis	
		Mean (±SD)	Mean (±SD)	Source of variation	p value
2.69 (±0.48)	F	2.778 (±0.43)	2.750 (±0.46)	Growth condition	0.000
	I	2.412 (±0.59)	2.563 (±0.50)	Ration	1.000
	S	1.556 (±0.62)	1.556 (±0.73)	Interaction	0.895



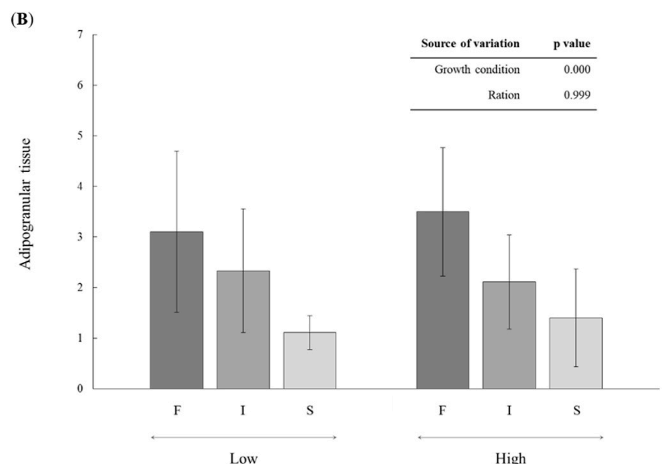


Fig. 6. Mean - values (±SD) of the semi-quantitative scorings recorded for the (A) digestive gland atrophy index and (B) the adipogranular tissue in the three growing groups (F, I and S) under both food rations. In a) the initial value is shown on the left with comparative purposes. Intervals indicate standard deviation. On the right of both graphs, the p-values of the Kruskal-Wallis testing the effects of growth condition and experimental diet are shown.

(Table 4).

Since the activities recorded seem to vary along with filtration activity, a plot has been built for each of the enzyme activities as a function of CR in Fig. 7: (A) catalase; (B) glutathione S-transferase; and (C) cytochrome c oxidase. For the three enzyme activities, it is apparent that fast growers were the group that maintained their enzyme activities most constant, whereas both S and I growers changed them depending on the ciliary activity that each of the food rations brought with it: the activity of catalase enzyme doubled up in both cases at low food ration (i.e., when the ciliary activity is higher). On the contrary, the activity of cytochrome c oxidase went down about a 25% in the I mussels, and over 20% in the S growers. In the case of glutathione S-transferase, no effect was true for the ration factor (Table 4), and in fact, the three growing groups showed quite stable activity values.

Table 4
Summary of the two-way factor ANOVA testing the effect growth condition (F vs. I vs. S) and diet (Low vs. High) on the three enzyme activities measured in the gill. N = 10 each experimental group.

Enzyme activities	Source of variation		
	Growth condition	Ration	Interaction
Catalase	DF = 2 F = 25.07 p = 0.000	DF = 1 F = 28.66 p = 0.000	DF = 2 F = 7.73 p = 0.001
Glutathione S-transferase	DF = 2 F = 19.14 p = 0.001	DF = 1 F = 0.03 p = 0.002	DF = 2 F = 0.57 p = 0.340
Cytochrome c oxidase	DF = 2 F = 8.67 p = 0.001	DF = 1 F = 10.74 p = 0.002	DF = 2 F = 1.11 p = 0.340

4. Discussion

The present study aimed to establish the nature of the differences in the growth capacity among Mytilus galloprovincialis juveniles, determining the factors giving rise to the outstandingly high inter-individual differences in their growth rate (e.g., Labarta et al., 1997; Tamayo et al., 2016). The maintenance in the laboratory of juvenile mussels under identical environmental conditions has resulted in the segregation of groups of mussels that differ greatly in their growth rate, as observed in previous works (Bayne et al., 1999[a,b]; Prieto et al., 2018, 2020; Toro et al., 2004). This demonstrates that a period of three months is long enough to significantly differentiate growth groups: specimens with the highest growth rate (F) grew, on average, 3 times faster than those with the lowest growth rates did (S) (0.163 vs. 0.052 mm/day).

One of the main results that has been obtained is that feeding rates per unit mass are consistently two times higher in F than in S mussels, regardless of the food ration they are supplied with. At high food ration, though, the mean CR values are significantly reduced in both types of mussels. The reduction of filtering activity in response to particle concentration increase is a behavior that has been comprehensively addressed and interpreted in bivalves as a mechanism allowing the regulation of ingestion rate and gut passage time when the diet consists entirely of organic matter (Bayne et al., 1987; Navarro et al., 1992, 1994). The lack of differences in the regulatory response of fast and slow growers has also been previously reported in M. galloprovincialis (Prieto et al., 2020).

Fast-growing specimens that display a higher filtering activity did not undergo a significant rise in routine metabolic rate per body mass unit, even if bivalves are known to increase their metabolic expenditure when ingestion rates increase (Babarro et al., 2000; Bayne et al., 1999). This implies that in addition to a higher capacity to acquire food, F mussels also have higher metabolic efficiency and/or lower costs of food processing, fostering faster growth. These results utterly fit the framework drawn by previous studies in oysters (Bayne et al., 1999a,[b]; Pace et al., 2006; Tamayo et al., 2014; Toro et al., 1998), clams (Tamayo et al., 2011, 2013, 2015) and mussels (Prieto et al., 2018, 2020), which highlighted feeding rate and metabolic efficiency as the most important physiological traits promoting inter-individual growth differences.

Size-related intra-specific differences in CRs have been explained so

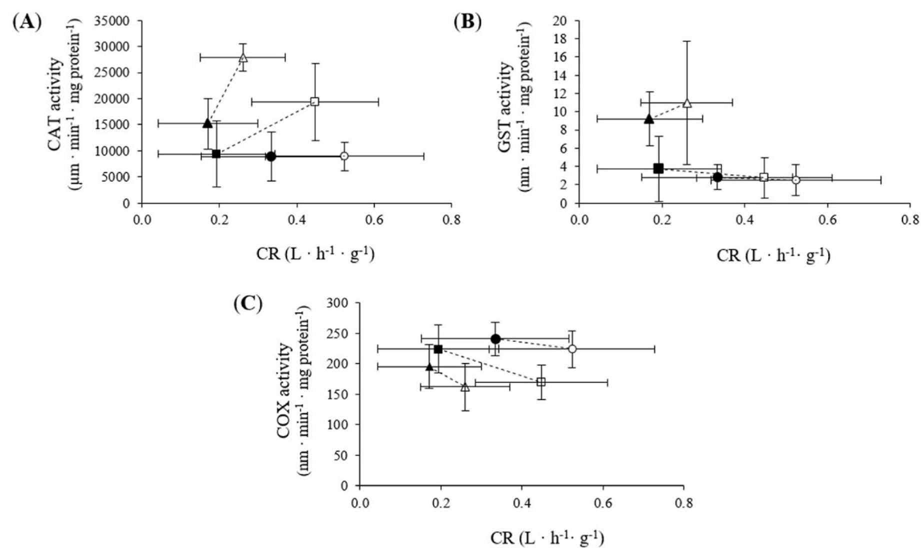


Fig. 7. Mean enzyme activities as a function of mean clearance rate values obtained for each growth condition group (fast growers: circles; intermediate growers: squares; slow growers: triangles) and ration (high concentrated ration: full symbols; low concentrated ration: empty symbols). (A) Catalase (B) Glutathione S-transferase and (C) Cytochrome c oxidase activities. Intervals indicate standard deviation.

far just by the corresponding differences in the gill-surface areas (e.g., Honkoop, 2003; Prieto et al., 2018). However, the underlying structural basis or cellular alterations have not been explored, which could also be affected. Histological evidence reported herein indicates precisely important functional differences in the gill structure that may determine the differential filtering rates between F and S mussels. The gill-structure scoring index developed in the present study is a good indicator of the density of lateral and latero-frontal cilia, and it is in good accordance with the other characteristics observed in each growth group. Moreover, it could be useful for integrative analysis of the growth assessment, as it provides additional information that is often overlooked in this type of analysis. In fact, lateral cilia are the primary drivers of water flow in M. galloprovincialis (Seo et al., 2014), and latero-frontal cilia are the ones in charge of particle capture and transporting (Riisgård and Larsen, 2010). Having a higher capacity for creating water currents, capturing particles and having a higher clearance rate overall lends F mussels, in terms of energy or growth, a higher physiological capacity than S mussels. Our results confirm a clear relationship between cilia density and growth rate, backed up by the over-expression of genes involved in tubulogenesis and ciliary activity reported for fast mussels (Prieto et al., 2019).

Cell damage, cilia structure and clearance rate data are in good agreement with the data obtained from the biochemical analysis. The activity levels of antioxidant enzymes are inversely related to the growth rate of mussels. Considering that the promotion of antioxidant enzyme production is a defensive response to removing ROS (Hayes and McLellan, 1999; Manduzio et al., 2005), higher oxidative stress in S mussels could be indicative of a higher ROS production in the gill cells. Differences in antioxidant enzyme production in this study between healthy fast and slow growing organisms that were not exposed to contaminants are as large as those found in pollution studies (e.g., Blanco-Rayón et al., 2019[a]; Gonçalves et al., 2020; Ozkan et al., 2017), turning the differences even more biologically meaningful. COX activity followed the opposite pattern of CAT and GST, indicating that S mussels have a lower usage of their aerobic metabolic pathways, hence suggesting a reduced aerobic metabolism. However, it should be noted that the COX activity was only measured in the gills, and it is only representative of the mitochondrial respiration, whereas the RMR is an estimation that takes into account all the tissues of the individual. This could explain a lack of concordance between COX activity and RMR values. In fact, COX activity is indicative of mitochondrial density and capacity (Blanco-Rayón et al., 2019[a]; Hüttemann et al., 2007; Morley et al., 2009; Sokolova, 2018). The reduced COX activity could imply a higher contribution of the anaerobic pathways in the metabolism of S mussels, which goes along with the up-regulation of genes involved in anaerobic metabolism that has been observed in the gills of this same mussel species (Prieto et al., 2019).

A reduced COX and enhanced antioxidant activities in S mussels suggest that inter-individual differences could stem from differences in the oxidative capacity of mitochondria. This could result in the gill of S mussels in a relatively lower capacity to couple oxygen consumption with ATP synthesis, which could be consistent with the recorded lower metabolic efficiency. Moreover, the greater cellular damage may impede the building of the ciliary mesh, leading to a lower filtering activity. Therefore, S mussels cannot confront the higher metabolic expenditure imposed by lowering particle concentration. Organisms that can obtain enough food, such as F and I mussels in this study, have much lower antioxidant activity, showing a positive relationship between antioxidant activity and poor nutritive status (González-Fernández et al., 2015). In fact, the activity differences between F, I and S mussels resemble those between starved and fed individuals in a study with the same mussel species (Blanco-Rayón et al., 2019[a]).

The inter-individual differences in food acquisition have not only been observed at low concentrations of suspended material, where a higher filtration capacity makes the difference when it comes to the food accessibility of the individual. At a high food ration, even if mean CR values are lower, F growers still display twice as much CR as S growers. Consequently, inter-individual differences in the physiological rates under high nutritional availability, in which having a better-developed filtration mechanism does not imply a functional advantage, indicates an underlying difference in the digestion and/or absorption capacity of the growing groups. Thus, assessment of the digestive tissue turns into a cornerstone to understanding the differences between growing groups, as the organisms must be able to use the ingested material.

The morphometric parameters included in the histological analysis reflect the digestive potential of the differentially growing organisms. In S mussels, the digestive diverticula were evidently reduced in number, and appeared scattered and surrounded by ample areas of connective tissue. A high CTD value indicates a loss of integrity of the digestive gland, which has been linked, among others, to poor nutritive status (Mújica et al., 2015). Actually, at a low food ration, the mean CTD value was significantly higher than at a high food ration. A lower proportion of

digestive diverticula means a lower proportion of effective tissue to process food. The low number of digestive diverticula that S mussels have appear to display a high level of atrophy typically characterized by an extreme thinning of the digestive tubules, where the digestive cells are overly fragmented. Degeneration of digestive cells has been reported in several mollusk species subjected to environmental stress (e.g., Syasina et al., 1997), and both atrophy and changes in the morphology of the digestive alveoli constitute a non-specific response to stressful environmental conditions (Benito et al., 2019; Kim et al., 2006). Regarding the cell type composition of the diverticula, S mussels showed a significantly higher density of basophilic cells. Under good physiological conditions, the digestive cells outnumber the basophilic cells, which is the case for F and I mussels in this study under both food rations. In S mussels, conversely, the relative occurrence of basophilic cells is apparently augmented due to digestive cell loss, a condition that is typical when mussels are subdued to stressful situations (Soto et al., 2002; Zaldibar et al., 2008). Therefore, even though S mussels have not been subdued to any stress, and they have been kept under the same conditions that F growers, they still possess degeneration traits that resemble those seen in healthy animals experimentally or naturally stressed. Compared to slow growers, fast growers display a better-equipped digestive tissue to process food altogether. The inherent differences between growing groups are so demonstrated, supporting the physiological data.

Understanding the process of inter-individual growth rate differentiation requires comparing the initial values and those recorded three months later, once the size-differentiation occurred, as suggested by Fuentes-Santos et al. (2018). Initial values of gill appearance are among the highest scores, whereas digestive tissue atrophy, CTD and Vv_{BAS} are among the lowest, thus indicating an optimal initial condition. Indeed, gonadal development was observed in the samples taken for the initial determination, which resulted in a massive spawning event after a few days in the laboratory. After laboratory conditioning, mussels reduced oxygen consumption, indicating a general improvement of the energy balance, a reduction that could be linked to the loss of gonadal tissue. It cannot be discarded as well a higher RMR at the beginning due to the acclimation to the laboratory. However, only F and I mussels increased their CR values. Histological analysis revealed that while F mussels maintained relatively similar values to the initial ones, S mussels have gill and digestive tissues that at some point started deteriorating. Actually, not only somatic growth was notably diminished, but also the accumulation of energy reserves: while in almost all F mussels adipogranular tissue was observed in the mantle (and even between the digestive diverticula), S mussels did not show such accumulation. In fact, the near absence of adipogranular tissue in S mussels was utterly independent of the ration, which goes along with the aforementioned idea that not even a considerable increase in food concentration is sufficient for S mussels to make up for their deteriorated tissues. Although more studies should be performed to detail the underlying causes, the loss of functional digestive capacity in S mussels could be the consequence of the previously discussed constrain in the filtering activity or, alternatively, could stem from a cell-damage in the digestive gland similar to that observed in the gill.

For the intermediate growers that show the behavior of the bulk of the population, a trend is apparent: when mussels are fed a low food ration, I individuals remain halfway between the values displayed by F and S mussels; whereas at a high food ration I mussels almost match the values attained by F growers. In some parameters, that unalike behavior is reflected statistically as an interaction. If the inter-individual differences recorded stemmed just from the S growers suffering a deterioration process, F and I growers should be expected to display similar performances and to have a similar appearance under the microscope. However, this is not the case. This may indicate that under conditions of high food availability, food ingestion of I mussels would not be compromised by their lower filtration ability. Hence, they would be even able to process and accumulate as many reserves as their F growing counterparts. It also brings forth the higher capacity that some mussels have to behave as F mussels even at low food rations.

The histological and biochemical characterization in this study complements the physiological data that points at differences in the food-acquisition rates and metabolic costs as determinants for inter-individual growth variability. The structural and functional differences found in S mussels suggest that their degenerated tissues or damaged cells impede the proper acquisition, digestion and absorption of food. This results, as a consequence, in the inability to face any nutritional event. On the contrary, F mussels stand out for their plasticity since, by keeping their histological and biochemical parameters virtually constant, they are able to obtain energy in the most efficient way under any ration.

This study serves as a starting point for field experiments testing the tissue and cellular organization of the different growing profiles in nature, including the effects that environmental conditions may impose. Furthermore, growth variability in nature is a major target of developing efficient aquaculture systems, and understanding the population dynamics will be relevant for such developments to happen.

CRediT author statement

Maitane Pérez-Cebrecos: Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing – original draft, Visualization. Daniel Prieto: Methodology, Validation, Investigation, Data Curation, Writing – Review and Editing. Esther Blanco-Rayón: Methodology, Validation, Data Curation, Writing – Review and Editing. Urtzi Izagirre: Conceptualization, Methodology, Resources, Writing – Review and Editing, Supervision, Funding acquisition. Irrintzi Ibarrola: Conceptualization, Methodology, Resources, Writing – Review and Editing, Supervision.

Funding

This work was supported by the Basque Government through Consolidated Research Groups fellowship (CBET group, IT810-B) and through the predoctoral fellowship of Maitane Pérez-Cebrecos.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The link to the data repository has been shared

References

Babarro, J.M., Fernández-Reiriz, M.J., Labarta, U., 2000. Metabolism of the mussel Mytilus galloprovincialis from two origins in the Ría de Arousa (north-west Spain). J. Mar. Biol. Assoc. U. K. 80, 865–872. https://doi.org/10.1017/S0025315400002848.

Bayne, B.L., Hawkins, A.J., 1997. Protein metabolism, the costs of growth, and genomic heterozygosity: experiments with the mussel Mytilus galloprovincialis Lmk. Physiol. Zool. 70, 391–402. https://doi.org/10.1086/515848.

Bayne, B.L., Hawkins, A.J.S., Navarro, E., 1987. Feeding and digestion by the mussel Mytilus edulis L. (Bivalvia). J. Exp. Mar. Biol. Ecol. 111, 1–22. https://doi.org/10.1016/0022-0981(87)90017-7.

Bayne, B.L., Hedgecock, D., McGoldrick, D., Rees, R., 1999a. Feeding behaviour and metabolic efficiency contribute to growth heterosis in Pacific oysters [Crassostrea gigas (Thunberg)]. J. Exp. Mar. Biol. Ecol. 233, 115–130. https://doi.org/10.1016/S0022-0981(98)00125-7.

Bayne, B.L., Svensson, S., Nell, J.A., 1999b. The physiological basis for faster growth in the Sydney rock oyster, Saccostrea commercialis. Biol. Bull. 197, 377–387. https://doi.org/10.2307/1542792.

Bayne, B.L., Thompson, R.J., Widdows, J., 1973. Some effects of temperature and food on the rate of oxygen consumption by Mytilus edulis L. In: Wieser, W. (Ed.), Effects of Temperature on Ectothermic Organisms. Springer), Berlin, Heidelberg, pp. 181–193. https://doi.org/10.1007/978-3-642-65703-0_15.

Benito, D., Ahvo, A., Nuutinen, J., Bilbao, D., Saenz, J., Etxebarria, N., Lekube, X., Izagirre, U., Lehtonen, K.K., Marigómez, I., Zaldibar, B., Soto, M., 2019. Influence of season-depending ecological variables on biomarker baseline levels in mussels (Mytilus trossulus) from two Baltic Sea subregions. Sci. Total Environ. 689, 1087–1103. https://doi.org/10.1016/j.scitotenv.2019.06.412.

Bignell, J.P., Dodge, M.J., Feist, S.W., Lyons, B., Martin, P.D., Taylor, N.G.H., Stone, D., Travalent, L., Stentiford, G.D., 2008. Mussel histopathology, effects of season, disease and species. Aquat. Biol. 2, 1–15. https://doi.org/10.3354/ab00031.

Blanco-Rayón, E., Guilhermino, L., Irazola, M., Ivanina, A.V., Sokolova, I.M., Izagirre, U., Marigómez, I., 2019a. The influence of short-term experimental fasting on biomarker responsiveness in oil WAF exposed mussels. Aquat. Toxicol. 206, 164–175. https://doi.org/10.1016/j.aquatox.2018.11.016.

Blanco-Rayón, E., Soto, M., Izagirre, U., Marigómez, I., 2019b. Enhanced discrimination of basophilic cells on mussel digestive gland tissue sections by means of toluidine-eosin staining. J. Invertebr. Pathol. 161, 29–39. https://doi.org/10.1016/j.jip.2019.01.001.

Brown, J.R., 1988. Multivariate analyses of the role of environmental factors and seasonal and site-related growth variation in the Pacific oyster. Crassostrea gigas. Mar. Ecol. Prog. Ser. 45, 225–236. https://doi.org/10.3354/meps045225.

Cartier, S., Pellerin, J., Fournier, M., Tamigneaux, E., Girault, L., Lemaire, N., 2004. Use of an index based on the blue mussel *(Mytilus edulis* and *Mytilus trossulus)* digestive gland weight to assess the nutritional quality of mussel farm sites. Aqua 241, 633–654. https://doi.org/10.1016/j.aquaculture.2004.08.015.

Claiborne, A., 1985. Catalase activity. In: Greenwald, R.A. (Ed.), CRC Handbook of Methods for Oxygen Radical Research. (Boca Raton, FL: CRC Press), pp. 283–284.

Fernández-Reiriz, M.J., Irisarri, J., Labarta, U., 2016. Flexibility of physiological traits underlying inter-individual growth differences in intertidal and subtidal mussels Mytilus galloprovincialis. PLoS One 11, e0148245. https://doi.org/10.1371/journal.pone.0148245.

Fuentes-Santos, I., Labarta, U., Fernández-Reiriz, M.J., 2018. Characterizing individual variability in mussel *(Mytilus galloprovincialis)* growth and testing its physiological drivers using Functional Data Analysis. PLoS One 13, e02059812. https://doi.org/10.1371/journal.pone.0205981.

Gaffney, P.M., Scott, T.M., 1984. Genetic heterozygosity and production traits in natural and hatchery populations of bivalves. Aqua 42, 289–302. https://doi.org/10.1016/0044-8486(84)90108-X.

Gonçalves, J.M., Rocha, T., Mestre, N.C., Fonseca, T.G., Bebianno, M.J., 2020. Assessing cadmium-based quantum dots effect on the gonads of the marine mussel *Mytilus galloprovincialis*. Mar. Environ. Res. 156, 104904 https://doi.org/10.1016/j.marenvres.2020.104904.

Gu, Z., Shi, Y., Wang, Y., Wang, A., 2011. Heritable characteristics in the pearl oyster *Pinctada martensii*: comparisons of growth and shell morphology of Chinese and Indian populations, and reciprocal crosses. J. Shellfish Res. 30, 241–246. https://doi.org/10.2983/035.030.0207.

Guilhermino, L., Lopes, M.C., Carvalho, A.P., Soared, A.M.V.M., 1996. Inhibition of acetylcholinesterase activity as effect criterion in acute test with juvenile. Daphnia magna. Chemosphere 32, 727–738. https://doi.org/10.1016/0045-6535(95)00360-6.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8.

Hawkins, A.J.S., Bayne, B.L., 1992. Physiological processes, and the regulation of production. In: Gosling, E. (Ed.), The Mussel Mytilus: Ecology, Physiology, Genetics and Culture. Elsevier Science Publishers, Amsterdam, pp. 171–222.

Hayes, J.D., McLellan, L.I., 1999. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defense against oxidative stress. Free Radic. Res. 31, 273–300. https://doi.org/10.1080/10715769900300851.

Hellou, J., Ross, N.W., Moon, T.W., 2012. Glutathione, glutathione *S*-transferase, and glutathione conjugates, complementary markers of oxidative stress in aquatic biota. Environ. Sci. Pollut. Res. 19, 2007–2023. https://doi.org/10.1007/s11356-012-0909-x.

Hildreth, D.I., Crisp, D.J., 1976. A corrected formula for calculation of filtration rate of bivalve molluscs in an experimental flowing system. J. Mar. Biolog. Assoc. 56, 111–120. https://doi.org/10.1017/S0025315400020476.

Honkoop, P.J.C., 2003. Physiological costs of reproduction in the Sydney rock oyster. Saccostrea glomerata. Oecologia 135, 176–183. https://doi.org/10.1007/s00442-002-1172-5.

Hüttemann, M., Lee, I., Samavati, L., Yu, H., Doan, J.W., 2007. Regulation of mitochondrial oxidative phosphorylation through cell signalling. Biochim. Biophys. Acta Mol. Cell Res. 1773, 1701–1720. https://doi.org/10.1016/j.bbamcr.2007.10.001.

Ibarrola, I., Etxeberria, M., Iglesias, J.I.P., Urrutia, M.B., Angulo, E., 2000. Acute and acclimated digestive responses of the cockle *Cerastoderma edule* (L.) to changes in the food quality and quantity. II. Enzymatic, cellular and tissular responses of the digestive gland. J. Exp. Mar. Biol. Ecol. 252, 199–219. https://doi.org/10.1016/S0022-0981(00)00235-5.

IBM Corp Released, 2017. IBM SPSS Statistics for Windows, Version 25.0. IBM Corp, Armonk, NY.

Jones, H.D., Richards, O.G., Southern, T.A., 1992. Gill dimensions, water pumping rate and body size in the mussel *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 155, 213–237. https://doi.org/10.1016/0022-0981(92)90064-H.

Kendall, B.E., Fox, G.A., 2002. Variation among individuals and reduced demographic stochasticity. Conserv. Biol. 16, 109–116. https://doi.org/10.1046/j.1523-1739.2002.00036.x.

Kim, Y., Ashton-Alcox, K.A., Powell, E.N., 2006. *Histological Techniques For Marine Bivalve Molluscs: Update*. Silver Spring. MD. NOAA Technical Memorandum, p. 76.

Kratzer, E., Tian, Y., Sarich, N., Wu, T., Meliton, A., Leff, A., Birukova, A.A., 2012. Oxidative stress contributes to lung injury and barrier dysfunction via microtubule destabilization. Am. J. Respir. Cell Mol. Biol. 47, 688–697. https://doi.org/10.1165/rcmb.2012-0161OC.

Labarta, U., Fernández-Reiriz, M.J., Babarro, J.M., 1997. Differences in physiological energetics between intertidal and raft cultivated mussels. Mytilus galloprovincialis. Mar. Ecol. Prog. Ser. 152, 167–173. https://doi.org/10.3354/meps152167.

Li, Q., Wang, Q., Liu, S., Kong, L., 2011. Selection response and realized heritability for growth in three stocks of the Pacific oyster *Crassostrea gigas*. Fish. Sci. 77, 643–648. https://doi.org/10.1007/s12562-011-0369-0.

Mallet, A.L., Haley, L.H., 1983. Growth rate and survival in pure population mating and crosses of the oyster. Crassostrea virginica. Can. J. Fish. Aquat. Sci. 40, 948–954. https://doi.org/10.1139/f83-121.

Manduzio, H., Rocher, B., Durand, F., Galap, C., Leboulenger, F., 2005. The point about oxidative stress in molluscs. Invertebr. Surviv. J. 2, 91–104.

Morley, S.A., Lurman, G.J., Skepper, J.N., Pörtner, H.-O., Peck, L.S., 2009. Thermal plasticity of mitochondria: a latitudinal comparison between Southern Ocean molluscs. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 152, 423–430. https://doi.org/10.1016/j.cbpa.2008.11.015.

Morton, B., 1983. In: Saleuddin, A.S.M., Wilbur, K.M. (Eds.), "Feeding and Digestion in Bivalvia" in *the Mollusca. Physiology Part 2*, ume 4. Academic Press, Inc.), pp. 77–82.

Mújica, M., Sokolova, I.M., Izagirre, U., Marigómez, I., 2015. Season-dependent effects of elevated temperature on stress biomarkers, energy metabolism and gamete development in mussels. Mar. Environ. Res. 103, 1–10. https://doi.org/10.1016/j.marenvres.2014.10.005.

Navarro, E., Iglesias, J.I.P., Ortega, M.M., 1992. Natural sediment as a food source for the cockle *Cerastodema edule* (L): effect of variable particle concentration on feeding, digestion and the scope for growth. J. Exp. Mar. Biol. Ecol. 156, 69–87. https://doi.org/10.1016/0022-0981(92)90017-5.

Navarro, E., Iglesias, J.I.P., Ortega, M.M., Larretxea, X., 1994. The basis for a functional response to variable food quantity and quality in cockles *Cerastodema edule* (Bivalvia, Cardiidae). Physiol. Zool. 67, 468–496.

Ozkan, D., Dagdeviren, M., Katalay, S., Guner, A., 2017. Multi-biomarker responses after exposure to pollution in the Mediterranean mussels *(Mytilus galloprovincialis* L.) in the Aegean coast of Turkey. Bull. Environ. Contam. Toxicol. 98, 46–52. https://doi.org/10.1007/s00128-016-1988-z.

Pace, D.A., Marsh, A.G., Leong, P.K., Green, A.J., Hedgecock, D., Manahan, D.T., 2006. Physiological bases of genetically determined variation in growth of marine invertebrate larvae: a study of growth heterosis in the bivalve *Crassostrea gigas*. J. Exp. Mar. Biol. Ecol. 335, 188–209. https://doi.org/10.1016/j.jembe.2006.03.005.

Pampanin, D.M., Camus, L., Gomiero, A., Marangon, I., Volpato, E., Nasci, C., 2005. Susceptibility to oxidative stress of mussels *(Mytilus galloprovincialis)* in the Venice Lagoon (Italy). Mar. Pollut. Bull. 50, 1548–1557. https://doi.org/10.1016/j.marpolbul.2005.06.023.

Pernet, F., Tremblay, R., Redjah, I., Sevigny, J.M., Gionet, C., 2008. Physiological and biochemical traits correlate with differences in growth rate and temperature adaptation among groups of the eastern oyster. Crassostrea virginica. J. Exp. Biol. 211, 969–977. https://doi.org/10.1242/jeb.014639.

Peters, R.H., 1983. The Ecological Implications of Body Size, vol. 2. Cambridge University Press, England. https://doi.org/10.1017/CBO9780511608551.

Prieto, D., Markaide, P., Urrutxurtu, I., Navarro, E., Artigaud, S., Fleury, E., Ibarrola, I., Urrutia, M.B., 2019. Gill transcriptomic analysis in fast- and slow-growing individuals of. Mytilus galloprovincialis. Aquac 511, 734242. https://doi.org/10.1016/j.aquaculture.2019.734242.

Prieto, D., Tamayo, D., Urrutxurtu, I., Navarro, E., Ibarrola, I., Urrutia, M.B., 2020. Nature more than nurture affects the growth rate of mussels. Sci. Rep. 10, 1–13. https://doi.org/10.1038/s41598-020-60312-y.

Prieto, D., Urrutxurtu, I., Navarro, E., Urrutia, M.B., Ibarrola, I., 2018. *Mytilus galloprovincialis* fast growing phenotypes under different restrictive feeding conditions: fast feeders and energy savers. Mar. Environ. Res. 140, 114–125. https://doi.org/10.1016/j.marenvres.2018.05.007.

Riisgård, H.U., Larsen, P., 2010. Particle-capture mechanisms in suspension-feeding invertebrates. Mar. Ecol. Prog. Ser. 418, 255–293. https://doi.org/10.3354/meps08755.

Robinson, W.E., 1983. Assessment of bivalve intracellular digestion based on direct measurements. J. Molluscan Stud. 49, 1–8. https://doi.org/10.1093/oxfordjournals.mollus.a065678.

Robinson, W.E., Michael, R.P., Langton, R.W., 1981. Variability of tubule types within the digestive glands of *Mercenaria mercenaria* (L), *Ostrea edulis* L., and *Mytilus edulis* L. *J. Exp. Biol. Ecol.* 54, 265–276. https://doi.org/10.1016/0022-0981(81)90161-1.

Romèo, M., Mourgaud, Y., Geffard, A., Gnassia-Barelli, M., Amiard, J.C., Budzinski, H., 2003. Multimarker approach in transplanted mussels for evaluating water quality in Charentes, France, coast areas exposed to different anthropogenic conditions. Environ. Toxicol. 18, 295–305. https://doi.org/10.1002/tox.10128.

Seo, E., Ohishi, K., Maruyama, T., 2014. Magnetic resonance imaging analysis of water flow in the mantle cavity of live. Mytilus galloprovincialis. J. Exp. Biol. 217, 2277–2287. https://doi.org/10.1242/jeb.101949.

Sokolova, Inna, 2018. Mitochondrial Adaptations to Variable Environments and Their Role in Animals' Stress Tolerance. Integr. Comp. Biol. 58 (3), 519–531. https://doi.org/10.1093/icb/icy017.

Soto, M., Zaldibar, B., Cancio, I., Taylor, M.G., Turner, M., Morgan, A.J., Marigómez, I., 2002. Subcellular distribution of cadmium and its cellular ligands in mussel digestive gland as revealed by combined autometallography and X-ray microprobe analysis. Histochem. J. 3*4*, 273–280. https://doi.org/10.1023/A:1023322423654.

Storey, K.B., 1996. Oxidative stress: animal adaptations in nature. Braz. J. Med. Biol. Res. 29, 1715–1733.

Syasina, I.G., Vaschenko, M.A., Zhandan, P.M., 1997. Morphological alterations in the digestive diverticula of *Mizuhipecten yessoensis* (Bivalvia: pectenidae) from polluted areas of peter the great bay, sea of Japan. Mar. Environ. Res. 44, 85–98. https://doi.org/10.1016/S0141-1136(96)00104-3.

Tamayo, D., Azpeitia, K., Markaide, P., Navarro, E., Ibarrola, I., 2016. Food regime modulates physiological processes underlying size differentiation in juvenile intertidal mussels. Mytilus galloprovincialis. Mar. Biol. 163, 1–13. https://doi.org/10.1007/s00227-016-2905-z.

Tamayo, D., Ibarrola, I., Navarro, E., 2013. Thermal dependence of clearance and metabolic rates in slow-and fast-growing spats of manila clam *Ruditapes philippinarum*. J. Comp. Physiol. B 183, 893–904. https://doi.org/10.1007/s00360-013-0764-1.

Tamayo, D., Ibarrola, I., Cigarría, J., Navarro, E., 2015. The effect of food conditioning on feeding and growth responses to variable rations in fast and slow growing spat of the Manila clam *(Ruditapes philippinarum)*. J. Exp. Mar. Biol. Ecol. 471, 92–103. https://doi.org/10.1016/j.jembe.2015.05.017.

Tamayo, D., Ibarrola, I., Urrutia, M.B., Navarro, E., 2011. The physiological basis for inter-individual growth variability in the spat of clams *(Ruditapes philippinarum)*. Aqua 321, 113–120. https://doi.org/10.1016/j.aquaculture.2011.08.024.

Tamayo, D., Ibarrola, I., Urrutxurtu, I., Navarro, E., 2014. Physiological basis of extreme growth rate differences in the spat of oyster *(Crassostrea gigas)*. Mar. Biol. 161, 1627–1637. https://doi.org/10.1007/s00227-014-2447-1.

Tamura, T., Yasuda, N., Shakuo, T., Kashiwagi, A., Martyn, J.A.J., Yokoyama, M., Yasuhara, S., 2020. Microtubule defect involved in 'mitophagy resistance' under subacute oxidative stress - potential mechanism for cellular inflammation. bioRxiv. https://doi.org/10.1101/2020.02.26.966234.

Toro, J.E., Vergara, A.M., 1998. Growth and heterozygosity in a 12-month-old cohort of *Ostrea chilensis* obtained by mass spawning in the laboratory. Mar. Ecol. 19, 311–323. https://doi.org/10.1111/j.1439-0485.1998.tb00470.x.

Toro, J.E., Alcapan, A.C., Ojeda, J.A., Vergara, A.M., 2004. Selection response for growth rate (Shell height and live weight) in the Chilean blue mussel *(Mytilus chilensis* Hupe 1854). J. Shellfish Res. 23, 753–758.

Vahl, O., 1973. Pumping and oxygen consumption rates of *Mytilus edulis* L. of different sizes. Ophelia 12, 45–52. https://doi.org/10.1080/00785326.1973.10430118.

Vincenzi, S., Mangel, M., Crivelli, A.J., Munch, S., Skaug, H.J., 2014. Determining individual variation in growth and its implication for life-history and population processes using the empirical Bayes method. PLoS Comput. Biol. 10, e1003828 https://doi.org/10.1371/journal.pcbi.1003828.

Weibel, E.R., 1979. Stereological Methods: Vol 1. Practical Methods for Biological Morphometry. Academic Press, London.

Zaldibar, B., Cancio, I., Marigómez, I., 2008. Epithelial cell renewal in the digestive gland and stomach of mussels: season, age and tidal regime related variations. Histol. Histopathol. 23, 281–290. https://doi.org/10.14670/HH-23.281.

Zar, J.H., 2010. Biostatistical Analysis, *fifth ed.* Pearson Prentice Hall, Upper Saddle River, New Jersey.

Zhang, J., Li, Q., Liu, S., Yu, H., 2018. The effect of temperature on physiological energetics of a fast-growing selective strain and a hatchery population of the Pacific oyster *(Crassostrea gigas)*. Aquacult. Res. 49, 2844–2851. https://doi.org/10.1111/are.13747.

Zhang, X., Ye, B., Gu, Z., Li, M., Yang, S., Wang, A., Liu, C., 2021. Comparison in growth, feeding, and metabolism between a fast-growing selective strain and a cultured population of Pearl oyster. Front. Mar. Sci. 8, 770702 https://doi.org/10.3389/fmars.2021.770702.