INFLUENCE OF STOCKING DENSITY ON GROWTH OF MUSSELS (*MYTILUS GALLOPROVINCIALIS*) IN SUSPENDED CULTURE

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Abstract

Crowding conditions in bivalve populations cause intraspecific competition processes, resulting in individual growth reduction. In aquaculture, density is usually maximized to obtain a greater commercial yield. Commercial farms provide an ideal scenario for studying the effect of density on mussel growth in suspended culture systems. In this study, different growth indicators for *Mytilus galloprovincialis* (growth rates, length and weight growth curves and size frequency distributions) were measured along a cultivation density gradient. Ropes cultured at different densities (220, 370, 500, 570, 700, 800 and 1150 ind/m) were hanged from a commercial raft and growth indicators were monitored monthly over the second phase of traditional culture in Galicia, from thinning-out to harvest (April to October 2008). A negative effect of density on individual growth was observed. Individuals cultured at lower densities presented higher growth rates and consequently reached greater weight and length values at the end of the experimental period than those cultured at higher densities. Differences in growth related to the cultivation density may suggest differences in intraspecific competition for limiting resources (space/food). Effects of density on growth started after 4 months of culture (August) when individuals reached sizes around 66 ± 1.3 mm. The increase in size of individuals in a population implies an increment of their food and space requirements, which in turn intensifies intraspecific competition. This fact should be considered in aquaculture management, since higher densities could be supported without effects on growth performance if cultured mussels are limited to a lower size.

Key words: crowding conditions, mussel growth, intraspecific competition, cultivation density, mussel culture and aquaculture management.

1. Introduction

The gregarious behavior characteristic of many benthic suspension-feeding invertebrates such as bivalves is associated with certain advantages including protection from predators (Bertness and Grosholz, 1985; Lin, 1991 and Reimer and Tedengren, 1997), reproductive success (Okamura, 1986) and optimization of hydrodynamic regimes leading to a higher flux of seston (Gibbs et al. 1991). However, high population densities may lead to food and space limitations inducing intraspecific competition phenomena (Alvarado and Castilla, 1996; Boromthanarat and Deslous-Paoli, 1988; Fréchette et al., 1992; Gascoigne et al., 2005; Guíñez and Castilla, 1999; Mueller, 1996; Okamura, 1986; Taylor et al. 1997). Intraspecific competition for limiting resources is usually reflected in growth reductions at the individual level (Alunno-Bruscia et al., 2000; Boromthanarat and Deslous-Paoli, 1988; Gascoigne et al., 2005; Guíñez and Castilla, 1999; Kautsky, 1982; Newell, 1990; Parsons and Dadswell, 1992; Peterson and Beal, 1989; Scarrat, 2000). Furthermore, as population density increases, intraspecific competition can also cause density-dependent mortality (Griffiths and Hockey, 1987; Richardson and Seed, 1990; Stillman et al., 2000; Stiven and Kuenzler, 1979). This mechanism, known as “self-thinning”, can regulate the size of the population regarding to the available resources (Westoby, 1984; Yoda et al., 1963).

In bivalves, crowding conditions were shown to have negative impacts on growth due to spatial limitations, inducing shell distortion (Bertness and Grosholz, 1985) or density-dependent migration (McGrorty and Goss-Custard, 1995). Moreover, physical interference between neighbours can result in restrictions to valve opening and thus clearance rate, which in turn cause reductions in feeding and mussel growth (Jørgensen et al. 1988). In addition, the large filtering capacity of mussels can cause depletion of seston particles in the water column and food limitations in cultivation emplacements (Dolmer, 2000; Gibbs et al., 1991; Grant, 1996; Lesser et al., 1992; Mueller, 1996; Navarro et al., 1991; Smaal and van Stralen, 1990). The quantity of food available at local scale depends on mussel population density, seston availability and the hydrodynamic patterns (Dame and Prins, 1998). In areas with extensive mussel cultivation, the water renewal time can limit the seston regeneration (Álvarez-Salgado et al., 2008; Álvarez-Salgado et al., 2011) with the subsequent growth reduction. Furthermore, natural cycles of food availability associated with oceanographic processes such as coastal upwelling, may modulate intraspecific competition processes and their consequences in individual growth and survival (Figueiras et al., 2002).

Competition for space and food has been observed in both natural mussel beds and cultured mussel populations (Ceccherelli and Barboni, 1983; Fréchette and Lefaivre, 1990; Fréchette et

al., 1992; Mueller, 1996; Taylor et al., 1997). Aquaculture on suspended structures represents a particular case of aggregation where density of suspension-feeders is maximized to achieve a greater commercial yield and economic benefit. Inhibition in feeding and declines in growth and survival rates have been observed in areas with high density of cultivated mussels (Fréchette and Despland, 1999).

Galicia is one of the largest mussel farming producers in the world, where mussels are grown in culture ropes suspended from raft systems (Gosling, 2003; Labarta, 2004). The productivity is sustained by coastal upwelling and the circulation patterns in the Rías that together stimulate high primary production rates (Figueiras et al., 2002). Nevertheless, the detrimental effect of density on mussel growth is well known by mussel producers. Aside from a reduction in food availability, crowding also increases the risk of mussel dislodgement from the ropes and subsequent financial losses. In traditional mussel cultivation, mussel density on culture ropes is reduced in a process called “thinning-out”. The “thinning-out” is carried out after 4-7 months when mussels reached shell lengths of 40-50 mm and growth slows down. This process consists of detaching the individuals from the ropes and replacing them in order to reduce the density and homogenize the size distributions (Pérez-Camacho et al., 1991). Mussel farmers can thus control mussel density on the ropes in order to optimize growth and minimize cultivation time and product losses. Although this method requires considerable labor and financial investment, it is commonly employed in the Galician Rías, thereby demonstrating the importance of mussel density on growth and commercial yield.

Studies on the effect of stocking density on mussel growth in suspended culture are scarce (Lauzon-Guay et al., 2005a; Lauzon-Guay et al., 2006; Pérez-Camacho and Labarta, 2004) despite the obvious importance to the mussel industry. A better understanding of the effect of stocking density on mussel growth will enable more efficient management at rope, raft and, ultimately, ecosystem scale, allowing the implementation of carrying capacity models (Rosland et al., 2011). The aim of this study is to determine the effect of mussel density on growth in a suspended culture situation, using the commercial culture techniques commonly employed in the Galician Rías. For this purpose, several growth indicators (shell length and weight growth rates, growth curves and size frequency distributions) were analyzed on seven density treatments during the second phase of cultivation (from thinning-out to harvest).

2. Materials and methods

2.1. Experimental design

Experimental suspended culture of *Mytilus galloprovincialis* was performed on a raft located at a commercial aquaculture polygon (Lorbé) in the Ría de Ares-Betanzos (NW Iberian Peninsula) (Fig. 1). Different growth indicators of mussels were measured on suspended culture ropes along a cultivation density gradient (220, 370, 500, 570, 700, 800 and 1150 individuals per meter of rope; ind/m). Experimental culture lasted six months, covering the second phase of commercial mussel culture in Galicia, from thinning-out to harvest (April to October 2008). The experimental culture was carried out following commercial protocols and handling techniques usually employed for mussel culture in Galician Rías.

In April, a total of 24 ropes for each experimental density were randomly distributed over a commercial raft. Mussels employed in the experimental culture were obtained from adjacent long-lines and presented a homogeneous size distribution. Initial shell length (mean $\pm$ SD) of mussels was 48.78 ± 1.27 mm, total dry weight was 2.52 ± 0.18 g., and tissue and shell dry weight were 0.41 ± 0.03 and 2.11 ± 0.15 g., respectively. No significant differences in initial length or dry weight values were observed between density treatments (ANOVA; $p > 0.05$).

2.2. Mussel sampling

Four ropes per density treatment were sampled monthly, between 1 and 6m depth, during the experimental period (May to October). At each rope, a sample of known surface was scraped free of mussels. From each sample, the maximum length of the antero-posterior axis of a minimum of 250 individuals was measured for the calculation of the mean shell length ($\bar{L}$; mm). Length data were classified into 5 mm length classes in order to analyze the size frequency distribution of each sample. Subsamples of 15-20 mussels per rope, covering a range of 10 mm around the mean length ($\bar{L}$) were employed for total, tissue and shell dry weight (TDW, DWt and DWs) calculation. First, the adductor muscle was cut and the individuals were placed on their ventral edge on filter paper to remove internal water. After dissecting the tissue from the shell, both were dried at 110°C until constant weight was obtained, then soft tissue and shell were weighed separately to obtain DWt and DWs. The total dry weight was calculated as the sum of tissue and shell dry weights.

2.3. Environmental conditions

At each sampling, measurements of temperature (T, °C) and salinity (S) were made using a YSI 556MPS multiprobe system at 1 and 6 m depth. Water samples were collected at each sampling time to calculate the concentration of chlorophyll-a (Chl-a; $\mu\text{g l}^{-1}$) and suspended particulate matter (mg l^{-1}) including the organic and inorganic fraction.

Total particulate matter (TPM) and the organic (POM) and inorganic (PIM) fractions were determined gravimetrically. Three replicates of 1 l seawater per sampling date were filtered on pre-combusted (450°C for 4h) and pre-weighed 25 mm Whatman GF/C filters. Salts were removed by rinsing with isotonic ammonium formate (0.5 M). Filters were dried at 110°C for 24h and weighed to determine the TPM concentration. The filters were then ashed at 450°C for 4h to determine the inorganic fraction. The organic fraction was calculated by difference between the total and the inorganic fraction. The determination of chlorophyll-a concentration was performed by spectrophotometry following the method of Jeffrey and Humphrey (1975). Three replicates of 1 l seawater per sampling date were filtered on 25 mm Whatman GF/C filters. The filters were frozen at -20°C to facilitate cellular rupture and improve chlorophyll extraction. The extraction was carried out for 12h using 5ml of 90% acetone (SCOR-UNESCO, 1966). Thereafter, the solution was centrifuged at 4500 rpm at 10°C for 10 min to separate the chlorophyll extract from the filter remains. The concentration was quantified using the equation developed by Jeffrey and Humphrey (1975): $\text{Chl-a} = (11.85 (E_{664} - E_{750}) - 1.54 (E_{647} - E_{750}) - 0.08 (E_{630} - E_{750}) v) / V$, where Chl-a is the chlorophyll-a concentration ($\mu\text{g l}^{-1}$), E_{750} , E_{664} , E_{647} and E_{630} are the absorbances at 750, 664, 647 and 630 nm respectively, v is the volume of acetone used in the extraction (ml) and V is volume of filtered seawater (ml).

2.4. Data analysis

For each density treatment and sampling date, weight values corresponding to the mean shell length were estimated by linear regression of log-transformed shell length (L) vs. total, tissue and shell dry weight (TDW, DWt and DWs).

Two-way factorial analyses of variance (Zar, 1999) were used to test the effect of density treatment and sampling time on the mean shell length and weight values (L, TDW, DWt and DWs). Under normality (Shapiro-wilk test, $p\text{-value} > 0.05$) and homogeneity of variance (Levene test, $p\text{-value} > 0.05$) conditions, parametric ANOVA followed by a Tukey-HSD (Honest Significant Difference) test were performed. Otherwise, the Kruskal-Wallis nonparametric test two-way ANOVA on ranked data was applied followed by the Wilcoxon test for pair-wise comparison between groups.

Shell length growth curves were fitted to a Gompertz (G) model: $L_t = L_{\infty}(e^{-e^{-k(t-t')}})$, where L_t is shell length (mm) at time t (days), L_{∞} is the maximum size, k is the growth parameter indicator of the speed at which maximum size is attained (days^{-1}) and t' is the inflexion point of the curve (Ratkosky, 1990). Similarly, the growth curves of total, tissue and shell dry weight were fitted to a Gompertz model: $DW_t = DW_{\infty}(e^{-e^{-k(t-t')}})$, whose parameters are analogous to those for shell length growth curves. The Gompertz model parameters were estimated by non-linear regression, using the Levenberg-Marquardt algorithm and least squares as loss function. Comparisons between the estimated Gompertz model parameters for each density treatment were made using an extra sum of squares (Chen et al., 1992). This technique (Motulsky and Christopoulos, 2004) facilitates comparing growth curves parameters directly between density treatments using a set of pairwise contrasts, by an F statistical test: $F = ((RSS_s) - (RSS_i)) / (df_s - df_i) / (RSS_i / df_i)$, where RSS_s and RSS_i are the residual sum of squares of the curves fitted with and without a parameter shared, respectively, and df_s and df_i are their corresponding degrees of freedom.

Length and weight (total, tissue and shell) growth rates were calculated for the entire experimental period (May-October) and the Spring-Summer (May-August) and Summer-Autumn (August-October) periods. Growth rates were calculated in mm day^{-1} and g day^{-1} , respectively, as the difference between length and weight values at the beginning and the end of each period. One-way ANOVAs were used to determine the effect of density treatment on growth rates for these periods. A Tukey test was performed as post hoc test.

The effect of density on the size frequency distribution of mussels was tested after the first month of cultivation (May) and at the end of the experimental period (October). As normality and homogeneity assumptions were not met, a one-way non-parametric nested ANOVA with the random factor rope nested to density was applied. However, as differences between ropes within each density were not found ($p\text{-value} > 0.05$), the nested effect was removed and a Kruskal-Wallis test, followed by a Wilcoxon post-hoc test were performed.

All data analyses were performed using the statistical software STATISTICA 6.0.

3. Results

3.1. Environmental conditions

Temperature during the experimental period ranged between minimum values of 13.5°C in April and maximums around 19.5°C in July/August. Salinity varied within a narrow range (34.0-35.9) and presented the lowest values during Spring, probably associated with the highest continental runoff. Chlorophyll-a concentration presented minimum values during Winter and tended to increase during Spring and Summer periods, showing several peaks in Spring, Summer and Autumn (Fig. 2A). The presence of a persistent phytoplankton bloom at the beginning of July corresponds to the highest peak in chl-a, TPM and POM (Fig. 2A-C).

3.2. Growth temporal evolution

The significant density×time interaction found for all the growth parameters evaluated (Table 1) revealed a different growth temporal evolution among density treatments (Fig. 3). There were no significant differences in any of the parameters studied (L, TDW, DWt and DWs) among density treatments during the first months of the experimental culture (May and June) ($p>0.05$; Fig. 3). However, in the following months an inverse relationship between size/weight mean values and density treatment was observed (Fig. 3), with the exception of August where the differences in growth indicators showed no relationship with density.

Significant increases in length (L), total dry weight (TDW) and shell dry weight (DWs) were observed from May to September for all treatments, except for the highest mussel density (1150 ind/m) where growth ceased in August (Fig. 3). Tissue dry weight (DWt) increased significantly from May until August, then growth ceased in all the density treatments (Fig. 3).

3.3. Size frequency distribution

Throughout the experimental period the size frequency distributions of all densities fitted a unimodal curve. No differences were observed in the size frequency distributions among experimental densities after the first culture month (May; Kruskal-Wallis test; $p\text{-value} > 0.05$). At harvest (October), size distribution showed a leftward displacement as density increased (Fig. 4 and Table 2), where the 220 ind/m density treatment presented the greatest mean shell length and the 1150 ind/m the lowest (Table 2).

3.4. Gompertz growth curves

A gradual decrease in mussel asymptotic size, total, tissue and shell weight as density increases was observed (Table 3).

Asymptotic sizes (L_{∞}) were significantly different between extreme density treatments ($p < 0.05$; Table 3). The lowest values were observed for the 1150 ind/m treatment (71.3 mm) and the highest in the 220 and 370 ind/m treatments (90.2 and 96.9 mm, respectively; Table 3 and Fig. 5A). Accordingly, in the lowest densities the maximum estimated length was 21 and 26.4% larger (for 220 and 370 ind/m, respectively) than the estimated for the highest density (1150 ind/m). Furthermore, we found significant differences in the growth factor (k) of shell length growth curves between the highest values observed for 370 ind/m mussels, and the lowest for 1150 ind/m ($p < 0.05$; Table 3).

The individuals cultured at the highest density (1150 ind/m) also reached a significantly lower total weight than those for the other densities ($p < 0.05$; Table 3 and Fig. 5B). Concurrently, the intermediate densities (570, 700 and 800 ind/m) reached lower total weights than those for 220 and 370 ind/m ($p < 0.05$; Table 3). This implies differences up to 35.8% in the maximum total weight estimated between the extreme density treatments (1150 and 220-370 ind/m). In addition, significant differences related to density were observed at the inflexion point (t^*) of TDW growth curves, showing an earlier change from linear to asymptotic growth in the higher density treatments (Table 3).

In concordance, individuals cultured at higher densities (between 570 and 1150 ind/m) reached significantly lower estimated tissue weights than those cultured at lower densities (220 and 370 ind/m) ($p < 0.05$; Table 3 and Fig. 5C). Differences up to 22.5% in the estimated maximum tissue weight attainable by individuals were observed among extreme density treatments (1150 and 220-370 ind/m). Similarly, asymptotic shell weights ($DW_{s_{\infty}}$) were significantly different between the lowest values observed for the density of 1150 ind/m (8.9g) and the highest values for 220 and 370 ind/m mussels (15.4 and 17.0g, respectively; Table 3 and Fig. 5D). Therefore, in the lowest density treatments, individuals achieved a shell weight 41.9 and 47.4% higher, respectively, than those at the highest density.

3.5. Growth rates

A decrease in the growth rates with increasing culture density was observed ($p < 0.05$; Table 4) when the entire culture period was analyzed. Accordingly to the post hoc test results, the highest density (1150 ind/m) showed significantly lower length growth rates than those for 220 and 500 ind/m (Table 5). A similar trend was observed for weight growth rates, whereby the density of 1150 ind/m presented lower TDW rates than the densities of 220, 370, 500 and 700 ind/m

(Table 5). In concordance, the highest density treatment showed lower DWt growth rates than 220, 370 and 500 ind/m treatments and lower DWs growth rates than 220 and 370 ind/m (Table 5).

However, when the Spring-Summer and Summer-Autumn periods were analyzed separately (May-August and August-October, respectively) we observed that the differences between density treatments did not show a pattern related with density gradient until the second period, where a decrease of growth rates with increasing density was observed (Table 5). Specifically, the highest density (1150 ind/m) showed significantly lower shell length growth rates than the other densities. Similarly, the 1150 ind/m density treatment presented lower TDW growth rates than density treatments between 220 and 500 ind/m and lower DWs growth rates than the densities between 220 and 570 ind/m. Furthermore, differences in the DWt growth rates between densities were no longer observed when these two periods were analyzed separately (Table 4 and 5).

4. Discussion

The present study showed the negative effect of high population density on mussel growth in suspended culture. Significant differences in the asymptotic values of the growth curves were observed among the different cultivation densities in all the growth parameters studied. Individuals cultured at lower density reached significantly higher maximum length and weight values than those at higher densities. This suggested that lower growth is a result of stronger intraspecific competition at high density populations.

Our results agree with those of Xavier et al. (2007) who observed that the mean size of *M. galloprovincialis* in “mussel beds” was larger in sites with lower densities. Widman and Rhodes (1991) and Côté et al. (1993) also found a greater growth in shell and tissue weight at low densities for pectinids in suspended culture. These authors have suggested that this could be due to the individuals acquiring a greater proportion of limiting resources (food/space). This would stimulate growth and enhance survivorship, either directly, through a reduction of competitive pressure (Griffiths and Hockey, 1987) or indirectly, through an increase of growth rate to a larger size (Xavier et al., 2007), reducing the risk of predation (Branch & Steffani 2004; Griffiths and Hockey, 1987; Paine, 1974).

Significant differences between extreme densities were also observed in the growth factor (k) of the shell length growth curves, with highest values at lowest densities. Therefore, the individuals at lower densities, aside from reaching larger sizes, grow faster. Significant differences in the inflexion point (t^*) among densities were only observed in the total dry weight (TDW) growth curves, where individuals cultured at higher densities reached the inflexion point

321 faster than those cultured at lower densities, that is, growth was linear for less time and
322 asymptotic growth was reached sooner. These support ANOVA results on the mean individual
323 L, TDW and DWs, where growth in size and weight ceased earlier in the highest densities than
324 at the lowest (August c.f. September). This was also reflected in the leftward displacement of
325 the size frequency distribution with increasing culture density, leading to a slower growth and a
326 greater proportion of small individuals on the highest density treatments.

327
328 Over the entire experimental period (May-October) there was a negative effect of density on the
329 growth rates in size and weight of individuals. This is in agreement with previous studies on
330 *Mytilus* spp. (Alunno-Bruscia et al., 2000; Gascoigne et al. 2005; Parsons and Dadswell, 1992;
331 Scarrat, 2000). However, when growth rates were analyzed in two different periods (Spring-
332 Summer and Summer-Autumn), we observed that the differences associated to density
333 treatment were concentrated in the second culture period. During the first period (May-August)
334 there was no influence of density on growth. However, from August, when the individuals had
335 reached a considerable size (66.2 ± 1.3 mm), a reduction in growth rates at higher density
336 populations was observed. This may indicate that as individuals grow their requirements for
337 limiting resources increase and intraspecific competition becomes more pronounced.
338 Furthermore, ageing decreases the absorption efficiency and slows growth (Pérez-Camacho et
339 al., 2000) due to the achievement of the stationary phase of growth. These effects would be
340 intensified by the decrease in food availability during these months (August-October; Fig 2). In
341 summary, individual growth seems to intensify intraspecific competition, which in turn
342 establishes the limits on mussel coverage and biomass reached by the population.

343
344 Our results pointed out the influence of density on mussel growth in suspended cultivation
345 which seems to be related to competition for limiting resources (space/food). The effect of
346 stocking density on the growth rate of bivalves has been well-documented in several species
347 under different culture and environmental conditions. In most of these studies, a negative
348 relationship between growth rate and density has been established (Alunno-Bruscia et al., 2000;
349 Boromthanarat and Deslous-Paoli, 1988; Cigarría and Fernández, 1998; Côté et al., 1993;
350 Gascoigne et al., 2005; Kautsky, 1982; Newell, 1990; Peterson and Beal, 1989; Román et al.,
351 1999; Scarrat, 2000; Taylor et al., 1997; Waite et al., 2005). Nevertheless, other studies have
352 found limited evidence for such a negative effect on mussel growth (Fuentes et al., 2000;
353 Lauzon-Guay et al., 2005a,b; Mallet and Carver, 1991; Sénéchal et al., 2008). The lack of a
354 negative effect of density on growth has been explained by the use of insufficient crowded
355 populations (Lauzon-Guay et al., 2005a,b; Sénéchal et al., 2008) or the greater influence of
356 macroenvironmental factors in determining mussel growth, such as food availability or current
357 speed (Fuentes et al., 2000; Gascoigne et al., 2005; Mueller, 1996; Sénéchal et al., 2008). It has

also been speculated that density-dependent reduction in the number of mussels (i.e. dislodgements and mortality), would alleviate competitive pressures and could mask the effect of density on mussel growth (Alunno-Bruscia et al., 2000; Boromtharanat and Deslous-Paoli, 1988; Fréchette et al., 1992; Fuentes et al., 2000; Lauzon-Guay et al., 2005a,b; Maximovich et al., 1996), while laboratory experiments where densities were maintained constant, detected a negative effect of density on growth and condition index (Alunno-Bruscia et al., 2000). In the present study we have recorded a negative effect of density on growth even when experimental densities were not kept constant throughout the experimental period. Decreases in density were also positively related to density treatment and resulted in a greater mortality at elevated densities (Cubillo, 2012). This has also been observed in other studies in suspended culture (Fuentes et al., 1998; Fuentes et al., 2000; Lauzon-Guay et al., 2005a), and leads to the convergence of the densities with time that seems to mitigate the effect of density on individual growth.

In summary, the results from the present work have established for the first time the effect of stocking density on mussel growth in suspended culture applying the cultivation techniques used by mussel producers, during the period from thinning-out to harvest. Our study suggests that density influences the maximum size and weight attainable by individuals, as well as their growth rate. Although seasonal changes in food availability can modulate the effect of density on growth, lowering densities could reduce its detrimental effect along the culture. This is in concordance with previous results obtained using traditional cultivation techniques (thinning-out) in Ría de Ares-Betanzos, which suggested that adjusting mussel density to the characteristics of the cultivation area could yield an increase of 10-15% in biomass production and at least 20% in economic benefits (Pérez-Camacho and Labarta, 2004). In addition, our results showed an increased effect of intraspecific competition with mussel size. This fact should be considered in aquaculture management, since higher densities could be supported without effects on growth performance if the final culture product is limited to a lower size.

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Figure Captions

Fig. 1. Map of the Ría de Ares-Betanzos showing the topography of this inlet (contour lines -m-) and highlighting the commercial mussel culture areas (polygons) and the sampling station (●) of the present study. Modified from Álvarez-Salgado et al. (2011).

Fig. 2 Evolution of (A) chlorophyll a (Chl-a), (B) total particulate matter (TPM) and (C) particulate organic matter (POM), over the experimental period at 1 m (solid line) and 6 m (dashed line) depth.

Fig. 3. Evolution of mean (A) length (L; mm), (B) total dry weight (TDW; g), (C) dry tissue weight (DWt; g) and (D) shell weight (DWs; g) values for each density treatment throughout the experimental period.

Fig. 4. Size frequency distributions for the seven density treatments at harvest (October).

Fig. 5. Growth curves fitted to the Gompertz model for the seven density treatments over the experimental period (May to October 2008) in terms of (A) shell length, (B) total dry weight, (C) tissue dry weight and (D) shell dry weight. Growth curve parameters are shown in Table 3.

Fig. 1

A

B

C

Fig. 2

Fig. 3

Fig. 4

C

D

Fig. 5

Table 1 Two-way ANOVA results testing the influence of density treatment and sampling time on the mean values of shell length (L; mm), total dry weight (TDW; g), tissue dry weight (DWt; g) and shell dry weight (DWs; g) of mussels grown in suspended culture

Source of variation	d.f.	S.S.	M.S.	F	p value
Talla					
Density	6	198	33	21,16	< 2e-16***
Month	5	8792	1758.5	1126.42	< 2e-16***
Density:Month	30	187	6.20	4.00	2.3e-08***
Residuals	126	197	1.60		
TDW					
Density	6	40.30	6.72	20.07	2.3e-16***
Month	5	1276.4	255.29	762.88	< 2e-16***
Density:Month	30	26	0.87	2.59	1.2e-04***
Residuals	126	42.20	0.33		
DWt					
Density	6	13131	2189	5.30	6.7e-05***
Month	5	307100	61420	148.69	< 2e-16***
Density:Month	30	22356	745	1.80	0.013**
Residuals	126	52046	413		
DWs					
Density	6	24.90	4.16	25.41	< 2e-16***
Month	5	792.5	158.51	968.95	< 2e-16***
Density:Month	30	14	0.47	2.86	2.4e-05***
Residuals	126	20.60	0.16		

(*) p<0.1; (**) p<0.05; (***) p<0.001

Table 2 Mean differences between the size frequency distributions ($L_{\text{column}} - L_{\text{row}}$) of the different density treatments, at the end of the experimental period (October). Asterisks indicate p-values for the respective unilateral pair-wise Wilcoxon test

	220	370	500	570	700	800
370	2.31 **					
500	2.81**	0.25				
570	11.87***	8.59***	9.19***			
700	2.17*	-0.14	-0.40	-8.76***		
800	7.84***	4.89***	5.11***	-4.04***	5.06***	
1150	21.64***	17.48***	19.06***	9.73***	17.69***	13.79***

(*) p-value < 0.1, (**) p-value < 0.05, (***) p-value < 0.01

Table 3 Estimated parameters and determination coefficients of the shell length (L, mm) and weight growth curves (TDW, DWt and DWs, g) fitted to a Gompertz model, for the different densities under study. All parameters are statistically significant ($p < 0.001$). Significant differences between estimated parameters are shown with different letters ($p < 0.05$)

Density	Asymptotic value	k	t'	R ²
Shell length				
220	90.2 ^a	-0.007 ^{ab}	66.4	0.98
370	96.9 ^a	-0.005 ^a	63.3	0.97
500	80.0 ^{ab}	-0.010 ^{ab}	70.6	0.98
570	77.7 ^{ab}	-0.009 ^{ab}	50.9	0.97
700	79.8 ^{ab}	-0.010 ^{ab}	70.1	0.97
800	82.2 ^{ab}	-0.008 ^{ab}	55.3	0.96
1150	71.3 ^b	-0.015 ^b	66.6	0.91
Total dry weight				
220	15.6 ^a	-0.013	181.6 ^a	0.98
370	16.2 ^a	-0.011	190.2 ^a	0.98
500	13.6 ^{ab}	-0.014	176.8 ^{ab}	0.97
570	12.2 ^b	-0.014	169.1 ^{ab}	0.98
700	12.4 ^b	-0.017	166.6 ^{bc}	0.97
800	12.1 ^b	-0.014	172.4 ^{ab}	0.96
1150	10.4 ^c	-0.018	163.9 ^c	0.91
Tissue dry weight				
220	2.50 ^a	-0.030	167.1	0.85
370	2.53 ^a	-0.024	168.0	0.90
500	2.27 ^{ab}	-0.031	164.7	0.86
570	2.05 ^b	-0.033	161.3	0.88
700	2.14 ^b	-0.033	159.4	0.92
800	2.08 ^b	-0.028	164.9	0.94
1150	1.96 ^b	-0.034	165.1	0.73
Shell dry weight				
220	15.4 ^a	-0.009	204.8	0.98
370	17.0 ^a	-0.008	226.0	0.98
500	12.2 ^{ab}	-0.011	188.4	0.97
570	12.9 ^{ab}	-0.009	198.5	0.98
700	11.2 ^{ab}	-0.012	176.0	0.97
800	11.9 ^{ab}	-0.010	192.6	0.97
1150	8.9 ^b	-0.016	173.1	0.90

Table 4 One-way ANOVA results testing the influence of culture density on the shell length growth rate (mm day⁻¹) and dry weights growth rates, i.e. total, tissue and shell weight (TDW, DWt and DWs, respectively; g day⁻¹), for the entire culture period (May-October 2008) and for the May-August and August-October periods

MAY-OCTOBER	d.f.	S.S.	M.S.	F	p-value
Length	6	0.0034	0.0006	4.52	0.004**
TDW	6	0.0008	0.0001	5.21	0.002**
DWt	6	0.0001	0.0000	4.97	0.003**
DWs	6	0.0005	0.0001	4.30	0.006**
MAY-AUGUST					
Length	6	0.0050	0.0008	3.71	0.011**
TDW	6	0.0006	0.0001	2.72	0.041**
DWt	6	0.0001	0.00001	0.65	0.690
DWs	6	0.0004	0.0001	6.26	0.001***
AUGUST-OCTOBER					
Length	6	0.0379	0.0063	9.89	3.2e-05***
TDW	6	0.0052	0.0009	3.72	0.011**
DWt	6	0.0004	0.0001	1.68	0.176
DWs	6	0.0030	0.0005	4.48	0.005**

(*) p<0.1; (**) p<0.05; (***) p<0.001

Table 5 Growth rates (mean $\pm$ SD) of the different culture densities in terms of shell length (mm day⁻¹), total, tissue and shell weight (TDW, DWt and DWs; g day⁻¹), for the periods May-October, May-August and August-October. Different letters indicate significant differences between groups (p<0.05)

		220	370	500	570	700	800	1150
MAY-OCT	Mean L	0.143^a	0.135^{a,b}	0.146^a	0.117^b	0.137^{a,b}	0.131^{a,b}	0.115^b
	SD	0.015	0.009	0.004	0.007	0.021	0.007	0.005
	Mean TDW	0.057^a	0.054^a	0.053^a	0.046^{a,b}	0.052^a	0.049^{a,b}	0.039^b
	SD	0.009	0.003	0.004	0.003	0.006	0.004	0.005
	Mean DWt	0.011^a	0.011^a	0.012^a	0.009^{a,b}	0.010^{a,b}	0.010^{a,b}	0.007^b
	SD	0.002	0.001	4.0E-04	0.002	0.001	0.001	0.002
	Mean DWs	0.046^a	0.043^a	0.041^{a,b}	0.037^{a,b}	0.041^{a,b}	0.039^{a,b}	0.032^b
	SD	0.007	0.003	0.003	0.002	0.005	0.003	0.004
MAY-AUGUST	Mean L	0.173^{a,b}	0.154^{a,b}	0.181^a	0.143^b	0.178^a	0.167^{a,b}	0.180^a
	SD	0.018	0.018	0.012	0.004	0.024	0.007	0.012
	Mean TDW	0.072^a	0.059^{a,b}	0.066^{a,b}	0.057^b	0.066^{a,b}	0.062^{a,b}	0.063^{a,b}
	SD	0.004	0.010	0.007	0.002	0.005	0.008	0.003
	Mean DWt	0.021	0.017	0.020	0.018	0.017	0.018	0.018
	SD	0.004	0.006	0.005	0.002	0.003	0.003	0.001
	Mean DWs	0.051^a	0.042^{b,c}	0.046^{a,b,c}	0.038^c	0.049^{a,b}	0.043^{a,b,c}	0.045^{a,b,c}
	SD	0.003	0.004	0.002	0.002	0.002	0.005	0.004
AUGUST-OCT	Mean L	0.100^a	0.102^a	0.096^a	0.079^a	0.079^a	0.079^a	-0.012^b
	SD	0.024	0.015	0.015	0.017	0.031	0.018	0.043
	Mean TDW	0.036^a	0.048^a	0.035^a	0.031^{a,b}	0.031^{a,b}	0.031^{a,b}	0.000^b
	SD	0.021	0.011	0.012	0.008	0.010	0.020	0.018
	Mean DWt	-0.003	0.004	0.000	-0.004	0.000	-0.001	-0.009
	SD	0.008	0.008	0.006	0.002	0.002	0.007	0.007
	Mean DWs	0.038^a	0.044^a	0.035^a	0.035^a	0.031^{a,b}	0.032^{a,b}	0.009^b
	SD	0.015	0.006	0.009	0.006	0.010	0.013	0.012