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

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12 **Abstract**

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

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

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## 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; Guiñez 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; Guiñez 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

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

79

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

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

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## 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 ± 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 110ºC 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, ºC) 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; $\mu g l^{-1}$) 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 1l seawater per sampling date were filtered on pre-combusted (450ºC 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 110ºC for 24h and weighed to determine the TPM concentration. The filters were then ashed at 450ºC 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 1l seawater per sampling date were filtered on 25 mm Whatman GF/C filters. The filters were frozen at -20ºC 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 10ºC 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): 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 ($\mu g l^{-1}$), $E_{750}$, $E_{664}$, $E_{647}$ 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-value>0.05) and homogeneity of variance (Levene test, p-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.

180    Shell length growth curves were fitted to a Gompertz (G) model: $L_t = L_\infty(e^{-e^{(-k(t-t'))}})$, where $L_t$ is

181    shell length (mm) at time t (days), $L_\infty$ is the maximum size, k is the growth parameter indicator

182    of the speed at which maximum size is attained (days$^{-1}$) and t´ is the inflexion point of the curve

183    (Ratkoskwy, 1990). Similarly, the growth curves of total, tissue and shell dry weight were fitted

184    to a Gompertz model: $DW_t = DW_\infty(e^{-e^{(-k(t-t'))}})$, whose parameters are analogous to those for shell

185    length growth curves. The Gompertz model parameters were estimated by non-linear regression,

186    using the Levenberg-Marquardt algorithm and least squares as loss function. Comparisons

187    between the estimated Gompertz model parameters for each density treatment were made using

188    an extra sum of squares (Chen et al., 1992). This technique (Motulsky and Christopoulos, 2004)

189    facilitates comparing growth curves parameters directly between density treatments using a set

190    of pairwise contrasts, by an F statistical test: $F = ((RSS_s)-(RSS_i)/(df_s - df_i))/(RSS_i/df_i)$, where

191    $RSS_s$ and $RSS_i$ are the residual sum of squares of the curves fitted with and without a parameter

192    shared, respectively, and $df_s$ and $df_i$ are their corresponding degrees of freedom.

193

194    Length and weight (total, tissue and shell) growth rates were calculated for the entire

195    experimental period (May-October) and the Spring-Summer (May-August) and Summer-

196    Autumn (August-October) periods. Growth rates were calculated in mm day$^{-1}$ and g day$^{-1}$,

197    respectively, as the difference between length and weight values at the beginning and the end of

198    each period. One-way ANOVAs were used to determine the effect of density treatment on

199    growth rates for these periods. A Tukey test was performed as post hoc test.

200

201    The effect of density on the size frequency distribution of mussels was tested after the first

202    month of cultivation (May) and at the end of the experimental period (October). As normality

203    and homogeneity assumptions were not met, a one-way non-parametric nested ANOVA with

204    the random factor rope nested to density was applied. However, as differences between ropes

205    within each density were not found (p-value > 0.05), the nested effect was removed and a

206    Kruskal-Wallis test, followed by a Wilcoxon post-hoc test were performed.

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

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## 3. Results

*3.1. Environmental conditions*

Temperature during the experimental period ranged between minimum values of 13.5ºC in April and maximums around 19.5ºC 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-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 ($L_\infty$) 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 ($DWs_\infty$) 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

284 (Table 5). In concordance, the highest density treatment showed lower DWt growth rates than
285 220, 370 and 500 ind/m treatments and lower DWs growth rates than 220 and 370 ind/m (Table
286 5).
287 However, when the Spring-Summer and Summer-Autumn periods were analyzed separately
288 (May-August and August-October, respectively) we observed that the differences between
289 density treatments did not show a pattern related with density gradient until the second period,
290 where a decrease of growth rates with increasing density was observed (Table 5). Specifically,
291 the highest density (1150 ind/m) showed significantly lower shell length growth rates than the
292 other densities. Similarly, the 1150 ind/m density treatment presented lower TDW growth rates
293 than density treatments between 220 and 500 ind/m and lower DWs growth rates than the
294 densities between 220 and 570 ind/m. Furthermore, differences in the DWt growth rates
295 between densities were no longer observed when these two periods were analyzed separately
296 (Table 4 and 5).
297
298
299 **4. Discussion**
300
301 The present study showed the negative effect of high population density on mussel growth in
302 suspended culture. Significant differences in the asymptotic values of the growth curves were
303 observed among the different cultivation densities in all the growth parameters studied.
304 Individuals cultured at lower density reached significantly higher maximum length and weight
305 values than those at higher densities. This suggested that lower growth is a result of stronger
306 intraspecific competition at high density populations.
307 Our results agree with those of Xavier et al. (2007) who observed that the mean size of *M.*
308 *galloprovincialis* in "mussel beds" was larger in sites with lower densities. Widman and Rhodes
309 (1991) and Côté et al. (1993) also found a greater growth in shell and tissue weight at low
310 densities for pectinids in suspended culture. These authors have suggested that this could be due
311 to the individuals acquiring a greater proportion of limiting resources (food/space). This would
312 stimulate growth and enhance survivorship, either directly, through a reduction of competitive
313 pressure (Griffiths and Hockey, 1987) or indirectly, through an increase of growth rate to a
314 larger size (Xavier et al., 2007), reducing the risk of predation (Branch & Steffani 2004;
315 Griffiths and Hockey, 1987; Paine, 1974).
316 Significant differences between extreme densities were also observed in the growth factor (k) of
317 the shell length growth curves, with highest values at lowest densities. Therefore, the
318 individuals at lower densities, aside from reaching larger sizes, grow faster. Significant
319 differences in the inflexion point (t´) among densities were only observed in the total dry weight
320 (TDW) growth curves, where individuals cultured at higher densities reached the inflexion point

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

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

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

358  also been speculated that density-dependent reduction in the number of mussels (i.e.
359  dislodgements and mortality), would alleviate competitive pressures and could mask the effect
360  of density on mussel growth (Alunno-Bruscia et al., 2000; Boromtharanat and Deslous-Paoli,
361  1988; Fréchette et al., 1992; Fuentes et al., 2000; Lauzon-Guay et al., 2005a,b; Maximovich et
362  al., 1996), while laboratory experiments where densities were maintained constant, detected a
363  negative effect of density on growth and condition index (Alunno-Bruscia et al., 2000). In the
364  present study we have recorded a negative effect of density on growth even when experimental
365  densities were not kept constant throughout the experimental period. Decreases in density were
366  also positively related to density treatment and resulted in a greater mortality at elevated
367  densities (Cubillo, 2012). This has also been observed in other studies in suspended culture
368  (Fuentes et al., 1998; Fuentes et al., 2000; Lauzon-Guay et al., 2005a), and leads to the
369  convergence of the densities with time that seems to mitigate the effect of density on individual
370  growth.
371
372  In summary, the results from the present work have established for the first time the effect of
373  stocking density on mussel growth in suspended culture applying the cultivation techniques
374  used by mussel producers, during the period from thinning-out to harvest. Our study suggests
375  that density influences the maximum size and weight attainable by individuals, as well as their
376  growth rate. Although seasonal changes in food availability can modulate the effect of density
377  on growth, lowering densities could reduce its detrimental effect along the culture. This is in
378  concordance with previous results obtained using traditional cultivation techniques (thinning-
379  out) in Ría de Ares-Betanzos, which suggested that adjusting mussel density to the
380  characteristics of the cultivation area could yield an increase of 10-15% in biomass production
381  and at least 20% in economic benefits (Pérez-Camacho and Labarta, 2004). In addition, our
382  results showed an increased effect of intraspecific competition with mussel size. This fact
383  should be considered in aquaculture management, since higher densities could be supported
384  without effects on growth performance if the final culture product is limited to a lower size.
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639    **Figure Captions**

640

641    **Fig. 1.** Map of the Ría de Ares-Betanzos showing the topography of this inlet (contour lines -m-

642    ) and highlighting the commercial mussel culture areas (polygons) and the sampling station (●)

643    of the present study. Modified from Álvarez-Salgado et al. (2011).

644

645    **Fig. 2** Evolution of (A) chlorophyll a (Chl-a), (B) total particulate matter (TPM) and (C)

646    particulate organic matter (POM), over the experimental period at 1 m (solid line) and 6 m

647    (dashed line) depth.

648

649    **Fig. 3.** Evolution of mean (A) length (L; mm), (B) total dry weight (TDW; g), (C) dry tissue

650    weight (DWt; g) and (D) shell weight (DWs; g) values for each density treatment throughout the

651    experimental period.

652

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

654

655    **Fig. 5.** Growth curves fitted to the Gompertz model for the seven density treatments over the

656    experimental period (May to October 2008) in terms of (A) shell length, (B) total dry weight,

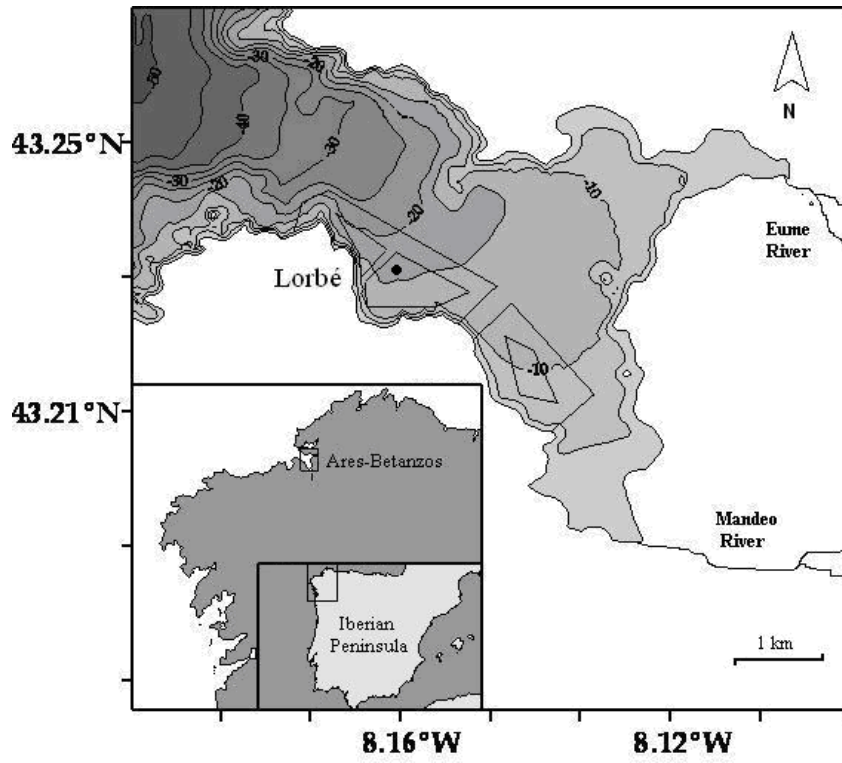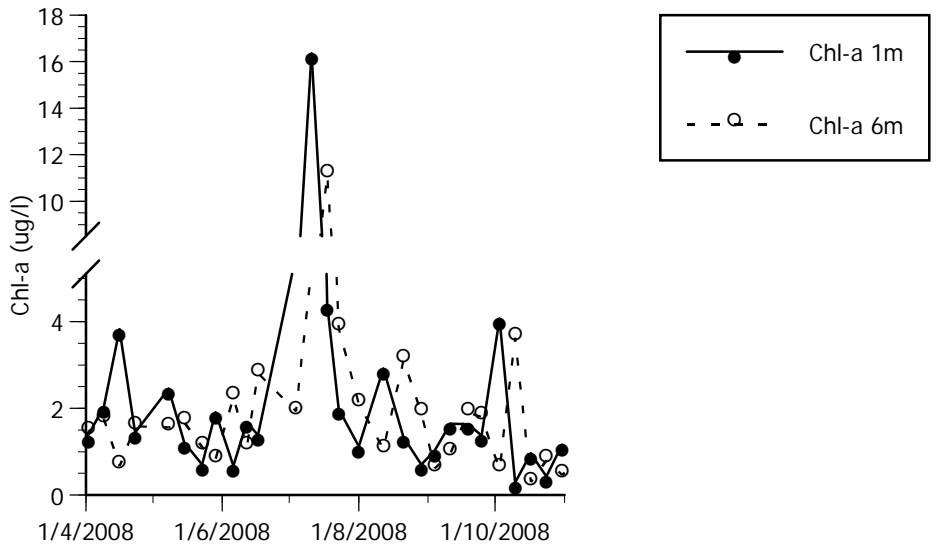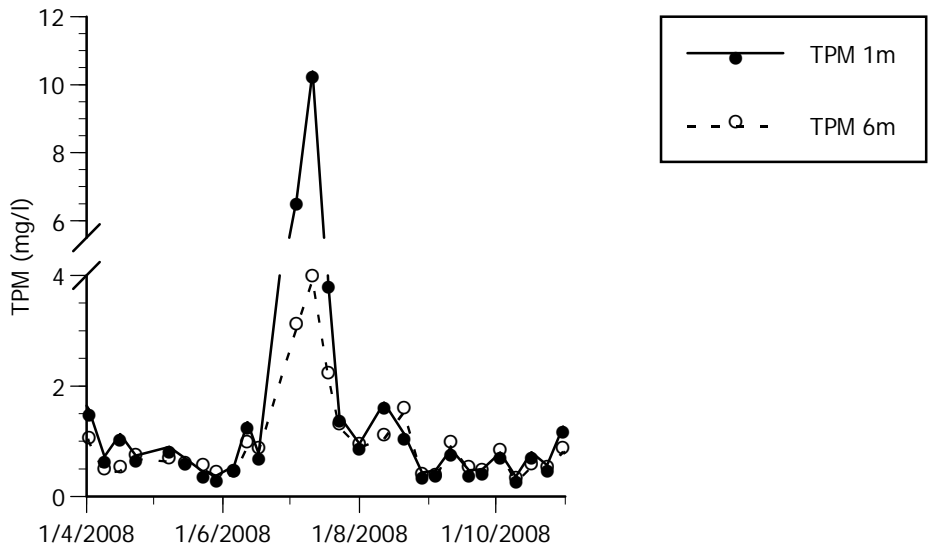657    (C) tissue dry weight and (D) shell dry weight. Growth curve parameters are shown in Table 3.
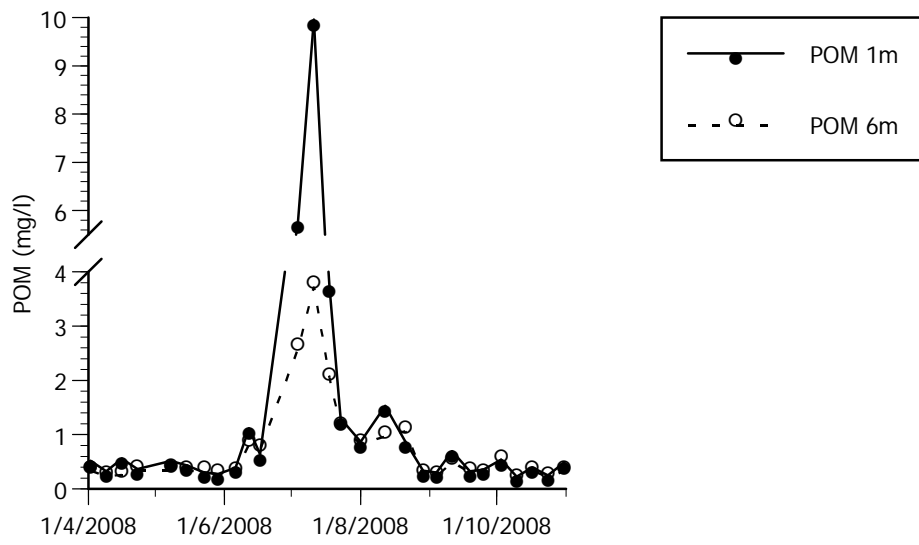
**Fig. 1**

C



**Fig. 2**
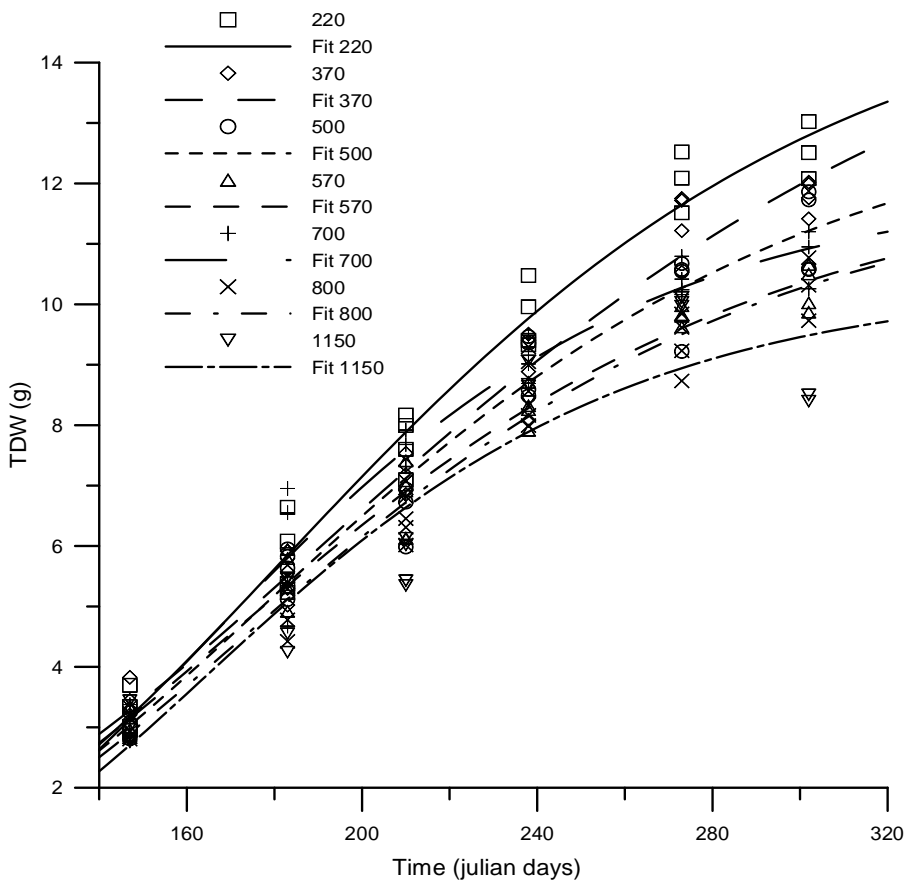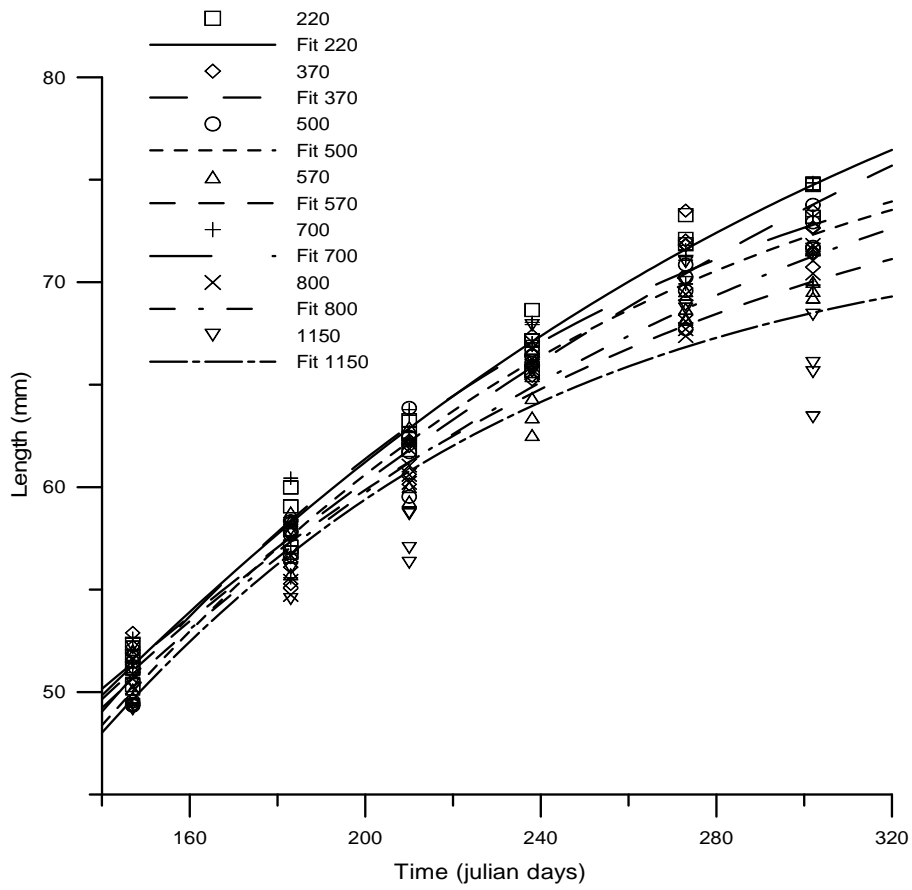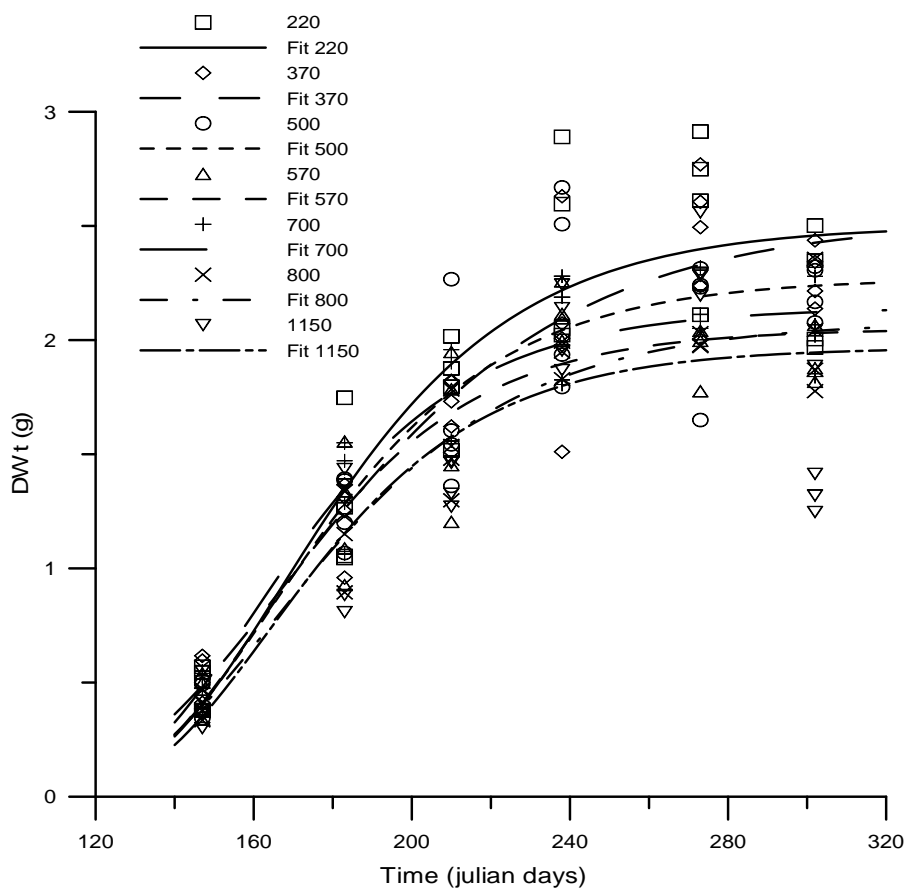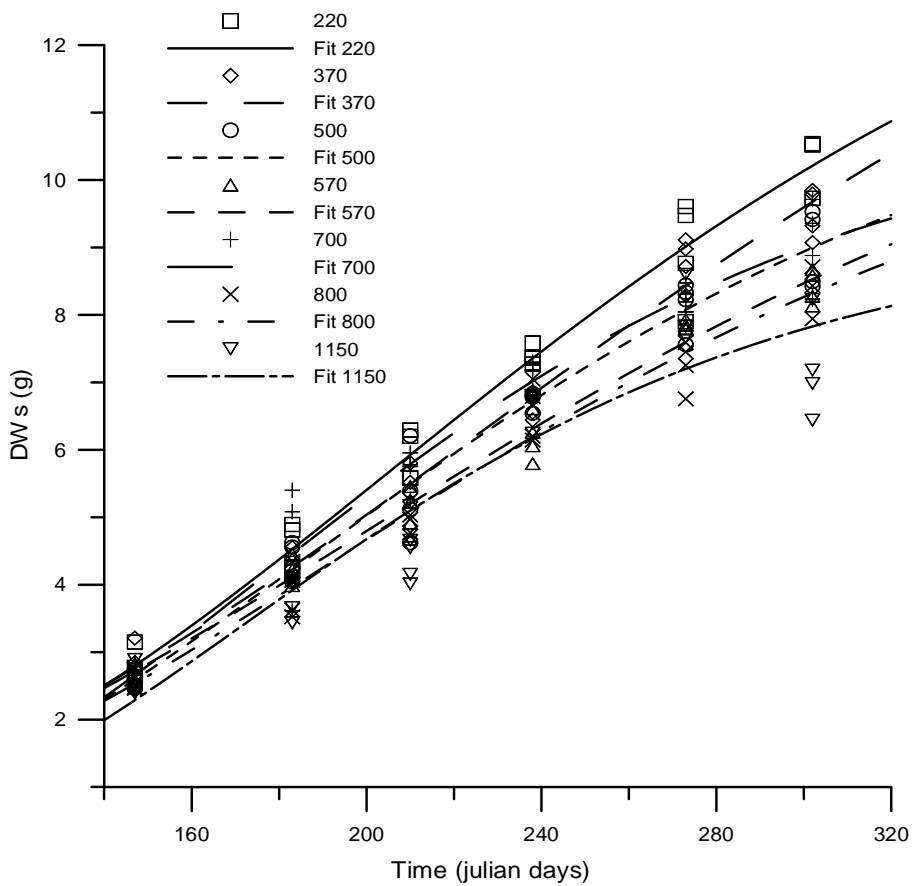
**Fig. 3**

**Fig. 4**

A



B
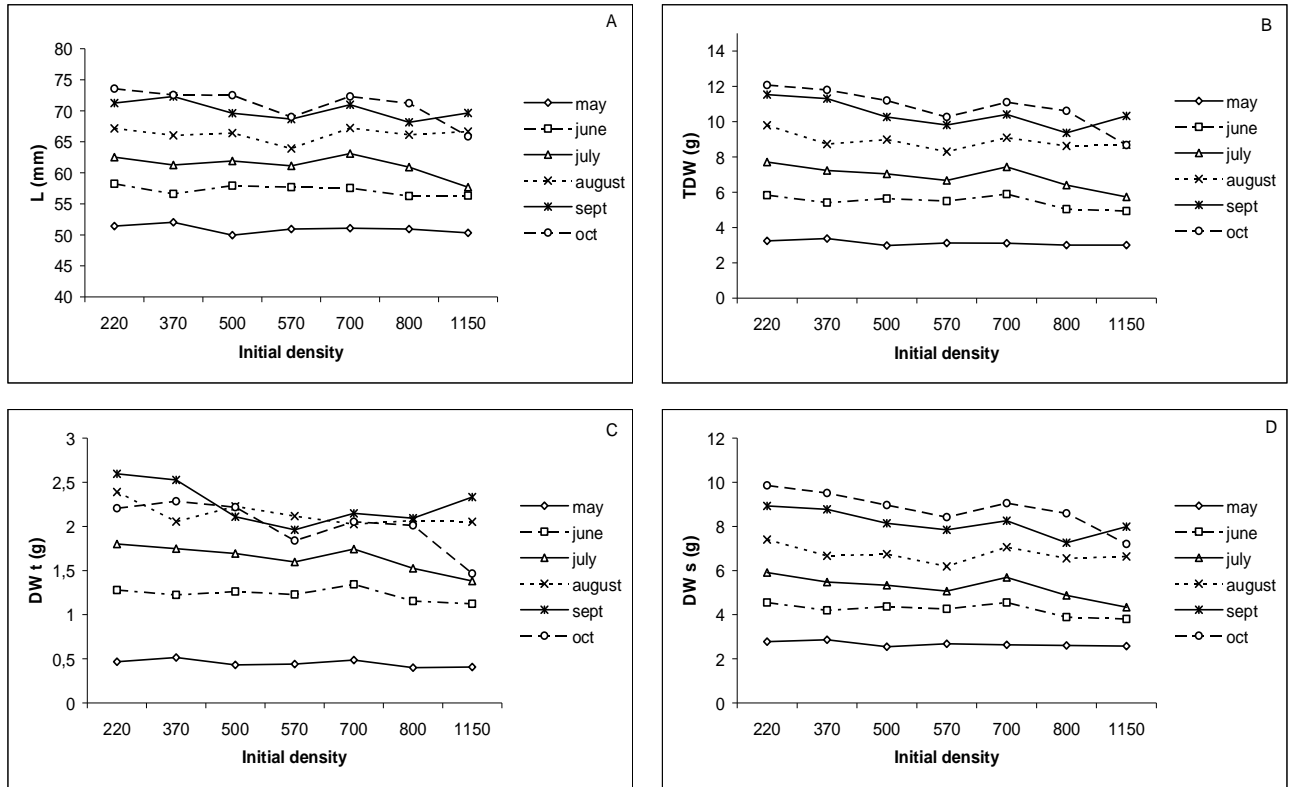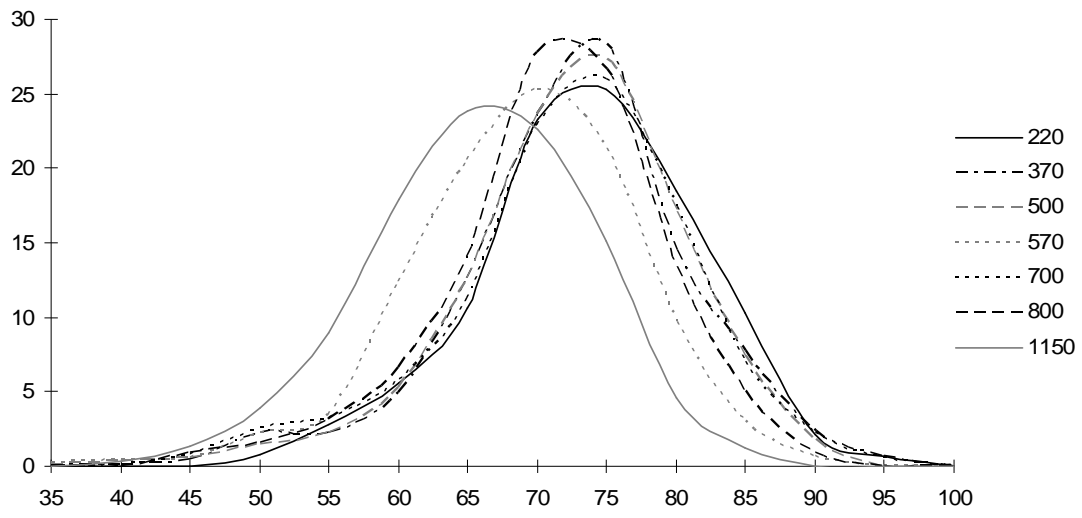
C



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 ($L_{column}$-$L_{row}$) 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 ± 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 |