**DENSITY-DEPENDENT EFFECTS ON MORPHOLOGICAL PLASTICITY OF
MYTILUS GALLOPROVINCIALIS IN SUSPENDED CULTURE**

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Abstract

Bivalve molluscs are characterized by high morphological plasticity in response to variations in local environmental conditions. In the present study, we evaluate this capacity in the mussel *Mytilus galloprovincialis* with regard to intra-specific competition caused by cultivation density. Suspended cultivation ropes at different initial densities (220, 370, 500, 570, 800 and 1150 individuals per meter of rope) were placed on a raft in the Ría Ares-Betanzos, following standard cultivation techniques. From May to October, covering the period from thinning out to harvest, various morphological indicators (length/width, length/height and height/width ratios) and allometric relationships (Volume-Length and Projected area-Length) in addition to energy distribution (dry tissue weight/dry valve weight) were analyzed. Differences in morphological indicators due to cultivation density were observed while no influence on the energy distribution was detected. From the second cultivation month (June), a decrease was recorded in the length/height ratio of individuals due to cultivation density. Similarly, there was also a decrease in the length/width ratio, although this decrease only became statistically significant from September. In addition, the allometric relationships studied (Volume-Length and Projected area-Length) suggested asymmetric competition processes. Differences in volume or projected area between densities were only detected at the end of the experimental culture (October) and only in the smaller individuals. These morphological adaptations can be understood as a strategy to mitigate the effects of intra-specific competition, palliating the consequences of physical interference at high densities.

Key words: morphological plasticity, *Mytilus galloprovincialis*, intra-specific competition, population density, industrial cultivation and physical interference.

1. Introduction:

Marine invertebrates exhibit a wide range of morphological variations in the natural environment. In bivalves, such variations can be caused by ontogenetic processes (Dickie et al. 1984; Stirling & Okumus 1994; Karayucel and Karayucel 2000) or a plastic response to environmental conditions, such as wave exposure (Akester and Martel 2000; Steffani and Branch 2003; Funk and Reckendorfer 2008), exposure to predators (Reimer and Tedengren 1996; Leonard et al. 1999; Caro and Castilla 2004) and population density (Seed 1968; Brown et al. 1976; Bertness and Grosholz 1985; Richardson and Seed 1990; Alunno-Bruscia et al. 2001; Briones and Guíñez 2005).

Cultivation density appears to be a particularly important environmental factor on the shell shape of the genus *Mytilus* (Seed 1968, 1973; Brown et al. 1976). Similar effects have also been observed in other species of molluscs, such as oysters (Tanita and Kikuchi, 1957; Chinzei et al. 1982) and clams (Ohba, 1956; Cigarria and Fernández, 1998). Alunno-Bruscia et al. (2001) noted that density influenced shell morphology and the condition index of *Mytilus edulis* cultivated in laboratory growth chambers. High densities lead to more elongated and less triangular shells with a lower height/length ratio (Coe 1946; Lent 1967; Brown et al. 1976; Richardson and Seed 1990; Briones and Guíñez 2005) and a lower width/length ratio (Alunno-Bruscia et al. 2001; Lauzon-Guay et al. 2005a). Bertness and Grosholz (1985) observed extreme distortions of more than 30° in the angle of the shells in *Geukensia demissa* at extremely high densities.

The previously cited studies suggest that morphological plasticity is a strategy to mitigate the effects of intra-specific competition at the individual level. Generally, these effects have been attributed to limitations in the availability of food, available substrate or the interaction of both of these factors (Fréchette and Lefaivre, 1990; Fréchette et al. 1992).

The negative effect of density on the growth of mussels has been widely studied (Alunno-Bruscia et al., 2000; Boromthanarat and Deslous-Paoli, 1988; Filgueira et al., 2008; Fréchette and Bourget, 1985a, b; Gascoigne et al., 2005; Grant, 1999; Guíñez and Castilla, 1999; Kautsky, 1982; Newell, 1990; Peterson and Beal, 1989). However, there are few studies on the effect of density on bivalve shell morphology in the field (Seed 1968; Bertness and Grosholz 1985; Richardson and Seed 1990; Briones and Guíñez 2005; Lauzon-Guay et al. 2005a). Of the aforementioned studies, only Lauzon-Guay et al. (2005a) focussed on the effect of density in a suspended culture system.

Mussels in suspended culture are exposed to different growing conditions than bottom or intertidal-cultivated mussels and wild or laboratory-grown mussels (due to differences in air exposure, wave action, temperature, food availability, currents, etc.). Therefore, the effect of crowding on morphological variations of individuals in suspended culture is not easy to predict from previous studies in different environments. Furthermore, the use of suspended culture systems allows the effect of crowding in the natural environment to be isolated, since it eliminates the interference of other variables, such as wave exposure, desiccation, etc.

Galicia is an important mussel producing region where most mussel farming occurs in suspended culture using rafts. In the traditional mussel culture as individuals increase their size, successive reductions in the density of mussels on the ropes are made. One of these reductions is called the “thinning out”. The "thinning out" process is carried out after 4-7 months, when the mussels have reached about 40-60 mm length and the weight of the ropes have increased significantly making mussel growth slower and heterogeneous. This process consists of detaching individuals from the ropes and re-attaching them in order to standardize the size and decrease the density on the ropes. The "thinning out" allows growers control of the density of mussels on the ropes in order to enhance their growth, while minimizing culture time and density-dependent losses (Pérez-Camacho and Labarta 2004). This method represents a considerable investment of working hours and labor costs, however, is a method commonly used in the Galician Rías. This highlights the importance of density on mussel growth and optimizing production in aquaculture.

The aim of this study is to analyze the effect of intra-specific competition on the morphological plasticity of *Mytilus galloprovincialis* (Lamarck 1819) in suspended culture by monitoring different morphological indexes in populations grown at different densities. For that purpose, we use commercial raft cultivation techniques that allow the manipulation of density in the natural field and facilitate the applicability of the results to the aquaculture industry.

2. Material and methods:

2.1. Experimental design

The study area was located in the raft polygon of Lorbé, in the Ría de Ares-Betanzos (NW of Spain), which is used intensively for the mussel *Mytilus galloprovincialis* cultivation (Fig. 1). The study employs the same standard culture and handling techniques as those used by the local industry. Mussels (*Mytilus galloprovincialis*) were placed on culture ropes at seven density levels (220, 370, 500, 570, 750, 800 and 1150 individuals/m of rope). The ropes were suspended

from a raft in April in order to study the period from thinning out to harvest (from May to October of 2008). Four ropes (replicates) were used by combination of 6 months and 7 density treatments (168 ropes), which were randomly distributed at the prow of the raft. Initial measures of shell length, weight and morphological characteristics are detailed in Table 1. Monthly samplings were performed until harvesting in October. Each month, a sample of known length of rope was taken from 4 ropes of each density treatment (28 ropes per sampling date) at an average depth of 2-4 m.

2.2. Sampling procedure

Individuals from each sample were counted to estimate density (individuals/m). The maximum length of the antero-posterior axis of a minimum of 200 individuals was measured for the calculation of the mean length ($\bar{L}$; mm). Sub-samples of 14 individuals per rope (56 mussels per density), covering a range of 30 mm around the average length, were used for morphometric measurements using callipers with a precision of 0.1mm. Length (L; mm) was calculated as the maximum distance of the antero-posterior axis. Height (H; mm) was considered as the maximum distance of the dorso-ventral axis and Width (W; mm) as the maximum lateral axis. These measurements were used to calculate the morphological W/L, H/L and H/W ratios.

Sub-samples of 15-20 mussels, covering a range of 10 mm around the mean length, were gathered from each rope replicate (60 to 80 mussels per density level) to calculate the total dry weight (TDW; g), tissue dry weight (DWt; g) and shell weight (DWs; g). After cutting adductor muscles and allowing intrapallial water to drain, mussels were dissected and both shell valves and soft tissues were weighed after drying at 110°C for 48 h. The total dry weight was calculated as the sum of the dry weights of tissue and shell. The condition index or meat yield was estimated as the ratio between the dry weight of soft tissues and the dry weight of the shell, according to the equation: $CI = (DWt / DWs)$.

Volume (V; ml) was calculated as the volume of water displaced after placing the mussel with the valves tightly shut in a vessel containing a known volume of seawater. To obtain the surface area occupied or projected area (S; cm²) by the mussels in the rope, dorsal photographs of 28 to 56 individual mussels from each experimental density were taken perpendicular to the antero-posterior axis, using a Nikon Coolpix 4500. The size range of mussel used was at least 15 mm around the mean length. The projected area on the substrate was calculated using image analysis software (ImageJ 1.37v).

We assumed an allometric relationship between the volume, projected area and the shell length of mussels. These allometries were established for each density and sampling period using

power equations of the form: $V = aL^b$ and $S = aL^b$, where a and b are the intercept and the allometric coefficient, respectively.

2.3. Statistical analysis

Analysis of covariance was applied to test the effect of initial density and sampling time on the intercepts and allometric coefficients for the log-transformed relationship Volume-Length and Projected area-Length (Snedecor and Cochran, 1989). Post hoc Tukey tests (t-tests) were used for pair-wise comparisons.

Analysis of variance (factorial ANOVA) was used to determine the effect of the initial density, time (month) and their interaction on each morphological ratio (W/L, H/L, H/W) and condition index (DWt/DWs). The homocedasticity assumption was met (Levene test, $p\text{-value} > 0.05$) for the three morphological variables. As ANOVA analysis is robust under slight deviations from the model hypothesis, the departure from normality found for some densities in each variable (Shapiro-Wilk test, $p\text{-value} < 0.01$) should not affect the results. For the condition index (DWt/DWs), heterogeneity of variances (Levene test, $p\text{-value} < 0.001$) in addition to non-normality for some densities (Shapiro-Wilk test, $p\text{-value} < 0.01$) were found, therefore a two way ANOVA on ranked data was applied. In cases where normality or homocedasticity assumptions were met, the Tukey HSD (Honest Significant Difference) test was used as a post-hoc, otherwise the Wilcoxon non-parametric test was performed.

Statistica 6.0 software was used for all analyses, considering a significance level of $\alpha=0.05$.

3. Results

3.1. Width-length ratio

Density treatment and time showed a significant effect on the W/L ratio (Table 2). Nevertheless, the significant interaction between those variables revealed a variable effect of density over the study period. At the beginning of the study, the width/length ratio (W/L) was similar for all densities (ANOVA; $p\text{-value} > 0.05$). From May to August a common tendency of an increase in this ratio was observed for all density treatments, without significant differences between densities (Fig. 2). After August, the W/L ratio continued to increase linearly in mussels cultivated at lower densities (220, 370 and 500 ind/m) (Table 3 and Fig. 2). For higher densities (570, 700, 800 and 1150 ind/m), the increase ceased and this ratio was maintained nearly constant until October, implying that these relationships were better described as second order polynomial functions (Table 3 and Fig. 2). Therefore, the lower densities (220 and 370 ind/m) reached significantly higher W/L values than the highest ones (800 and 1150) at the end of the experimental culture (September and October).

3.2. Height-length ratio

The H/L ratio was influenced by density treatment and sampling time, but not independently since the density $\times$ time interaction was significant ($p < 0.05$) (Table 2). At the start of the study (May), the height-length ratio presented similar values for all densities (ANOVA p -value > 0.05). There was a tendency towards a decrease in the H/L ratio with time for all densities, which was most pronounced toward the end of the cultivation period (Fig. 3). However, post hoc analysis showed that this decrease was only significant for the densities lower than 1150 ind/m. From the second month of cultivation (June), a negative effect of density on the H/L ratio was observed (Fig. 3). This tendency was maintained until the end, leading to more elongated shells in mussels cultivated at greater densities.

3.3. Height-width ratio

Density treatment and time showed a significant effect on the H/W ratio (ANOVA; $p < 0.05$; Table 2). The mussels always grew more in height than width ($H/W > 1$), but at decreasing rates, since the H/W decreased significantly with time (Fig. 4 and Table 4). Nevertheless, the significant interaction between density and time (ANOVA; $p < 0.05$; Table 2) revealed a variable effect of density over the study period. Accordingly, no significant effect of the initial density on the H/W ratio was detected, with the exception of August where a decrease of this ratio with increasing density was observed.

3.4. Energy distribution

The condition index was influenced by time (ANOVA; $p > 0.05$), while density treatment ($p = 0.259$) and the density-time interaction were not significant ($p = 0.492$; Fig. 5 and Table 2). There was a significant increase in meat yield from May to June. Such increase was maintained, though not significantly, until summer (July/August) when maximum values were reached (Fig. 5). Thereafter, the condition index decreased until the end of the study to values closed to those observed in May (Fig. 5).

3.5. Volume-Length relationships

Volume-Length relationships showed similar slopes between density treatments until October (ANCOVA; $F = 2.21$, $p = 0.042$). Differences observed between intercepts during that period (May-September) did not show a relationship with the density gradient. At the end of the experimental period (October) the pairwise comparisons t-test showed significant lower slopes for Volume-Length relationships for the 220 density treatment compared to the 800 and 1150 densities ($p < 0.05$). Similarly, the slopes observed for the allometric relationships for the 370 and 500 densities showed significantly lower values than that for the 800 density (Table 5).

However, the intercepts followed an inverse trend than the slope values, decreasing with an increase in initial density (t-test; $p < 0.05$; Table 5). The lowest densities produced a significantly lower increase in volume per size increment compared to the highest densities, which indicates a smaller difference in volume between the individuals of different sizes at lower density.

3.6. Projected area-Length relationships

Differences between densities in the slopes of the Projected area-Length relationships were only observed for the last month of cultivation (October) (ANCOVA; $F = 3.10$, $p = 0.007$). With regard to the intercepts, significant differences were observed among density treatments in every sampling ($p < 0.05$), but those differences did not follow a density gradient until the end of the study (October). In October, an inverse relationship between the intercept magnitude and the initial density was observed (Table 6). In addition, in this month there was a progressive decrease in the slopes related with the decrease in the initial cultivation density (Table 6). The pairwise t-test found significantly lower slopes for the lower density treatments ($p < 0.05$). Specifically, the 220 ind/m density showed significantly lower slopes than the densities of 700, 800 and 1150, whereas the 370 ind/m slope was lower than those of 800 and 1150 and that of 500 was lower than the slope for 800 ind/m.

4. Discussion

In our studies we observed a growth-related variation in the morphology of mussels. As for the majority of organisms, mussels exhibit gradual changes in their relative proportions as they increase their size. From a physiological perspective, these changes can be associated with the maintenance of optimal proportions (West et al. 1997; Banavar et al. 1999) or may reflect an adaptive response to changes in environmental conditions (Seed 1980; Seed and Suchanek 1992; Reimer et al. 1995; Akester and Martel 2000; Steffani and Branch 2003). The effect of growth on morphology is consistent in all the experimental densities evaluated in this study, showing a decrease in the H/L and H/W ratios and an increase in W/L along the study period. However, during the final month of cultivation these ratios tended to stabilize. Despite growth in all dimensions (length, width, height), changes in morphological ratios indicate a gradual change in the relative proportions of the mussels with time. In other words, the rate of growth in height decreases over time with respect to length or width, whereas the growth in width occurs at higher rates than growth in length. Due to this differential growth of shell dimensions, the larger (older) mussels have more elongated morphology where height exceeds width in ever decreasing proportions.

These results agree with previous studies on mussel growth and morphology where variations in mussel shape were attributed to age (Seed 1968; Brown et al. 1976; Alunno-Bruscia et al. 2001; Lauzon-Guay et al. 2005a). This allows us to isolate the effect of normal growth on morphology from that related to intra-specific competition. Our results agree with those of Seed (1968) who observed that the H/L and H/W ratios decreased with increasing size, whereas the W/L ratio increased. Similarly, Lauzon-Guay et al. (2005a) recorded a decrease in H/L and an increase in W/L with time. Alunno-Bruscia et al. (2001) also noted similar tendencies as the observed in the present study for W/L and H/W ratios, but an increase in H/L ratio with the increase in size. These discrepancies may be related to different experimental conditions (laboratory versus field), cultivation method (growth chambers versus suspended cultivation), or the growth period studied (juveniles -2cm- versus adults -4.62±3.8cm-).

The present study also showed a significant effect of cultivation density on various morphological indicators. From June onwards the mussels cultured at higher densities showed a decrease in the height-length (H/L) ratio and, from September, a decrease in the width-length ratio (W/L) was also observed. Thus, mussels cultivated at higher densities showed at the end of the study more elongated and narrower shells than those growing at low densities, which tend to be wider and higher. The density effect on both ratios (H/L and W/L) became more pronounced with time, showing the largest differences between densities at the end of the cultivation period. This may be related to the increasing limitations by food and space as the individuals grow and increase their requirements, which in turn increase intra-specific competition (Boromthanarat and Deslous-Paoli 1988; Marquet et al. 1990; Fréchette & Lefaivre 1995, Petraitis 1995; Alunno-Bruscia et al. 2000). However, the effect of density on the H/W ratio was only detected in August. This would suggest that the decrease in height and width of the mussels caused by intra-specific competition occurs evenly over the density gradient.

These results agree with previous studies that reported more elongated and less triangular mussels with a lower height/length ratio (Coe 1946; Lent 1967; Brown et al. 1976; Richardson and Seed 1990; Briones and Guíñez 2005) and a lower width/length ratio (Alunno-Bruscia et al. 2001; Lauzon-Guay et al. 2005a) at higher mussel densities. Together, this suggests that morphological plasticity is a strategy to mitigate the effects of intra-specific competition at the individual level (Seed 1968, 1973, Brown et al. 1976). There is a great controversy of the limiting factor that determines the appearance of intra-specific competition mechanisms in bivalves. Some authors suggest limitation by the concentration of available food (“exploitative competition”; Seed 1968; Alunno-Bruscia et al. 2001), whereas others attributed intra-specific competition mechanisms to physical spatial limitations (“interference competition”; Okamura 1986; Fréchette et al. 1992). The morphological changes observed in the mussels cultivated at

higher densities could be a strategy to reduce physical interference between individuals. Although it is not clear if physical interference has an effect on growth (Fréchette and Lefaiivre 1990; Fréchette and Despland 1999), such interference may cause a reduction in valve gaping, clearance rate and food uptake (Jørgensen et al. 1988). Mussels cultured at high densities and laterally constricted will encounter less restriction to valve opening (Lauzon-Guay et al. 2005a). The elongation of shells (more filiform shapes) might also enable mussels to position themselves more favourably to expose their inhalant siphons to better access for food (Sénechal et al. 2008), this might be part of the food competitive adaptation to culture, i.e., exploitative competition. The strategy of attaining more elongated shells seems, therefore, to be advantageous in high density environments and is a common characteristic in mussels grown in suspended culture (Lauzon-Guay et al. 2005a and present study) and in other cultivation methods such as laboratory tanks (Alunno-Bruscia et al., 2001) and “mussel beds” (Seed 1968).

Nevertheless, no significant effect of the cultivation density on the energy distribution between shell and tissue was observed at any time during the study period. This does not imply that the individuals cultivated at lower densities do not obtain a larger quantity of energy, but rather that the energy distribution between shell and tissue is maintained in the same proportion for all densities. In fact, higher asymptotic values have been achieved in the size and weight growth curves of mussels cultivated at lower densities (Widman and Rhodes 1991; Côté et al. 1993; Xavier et al. 2007 and Cubillo et al. submitted), as well as higher growth rates (Grant 1999; Alunno-Bruscia et al. 2000; Gascoigne et al. 2005 and Cubillo et al. submitted). Differences in the growth of tissue and shell between densities are proportional and not observable when combined in the condition index. Other studies (Mallet and Carver 1991; Alunno-Bruscia et al. 2001) observed a density effect on the energy distribution in mussels. However, the different allocation energy index used and the differences in the experimental design issues (laboratory culture chambers) could explain, respectively, the discrepancies with our results. Lauzon-Guay et al. (2005b) observed that prior to spawning mussels cultivated at higher density channelled a lower proportion of resources into tissue growth, with respect to those cultivated at lower densities. Nonetheless, these differences disappeared in post-spawning mussels, implying that the extra energy had been channelled into gonadal production (Lauzon-Guay et al. 2005b). This hypothesis is supported by Bayne et al. (1983) who observed a reduction in reproductive effort under environmental stress. This would also explain the lack of significant differences in the condition index between densities in this study, which took place after the main spawning period (spring) of this latitude (Villalba 1995; Snodden and Roberts 1997; Cáceres-Martínez 1998a, 1998b; Suárez et al. 2005; Peteiro et al. 2007). In general, the condition index (CI) is considered to be a weak indicator for detecting stress in bivalve molluscs (Guolan and Yong 1995; Hummel et al. 1996; Amiard et al. 2004; Duquesne et al. 2004; Lauzon-Guay et al.

2005a; Waite et al. 2005) because most of the variability in the CI is associated with fluctuations in the gametogenic/reproductive cycle and to seasonal variations in food availability (Hummel et al. 1996; Leung & Furness, 2001; Amiard et al. 2004; Boscolo et al. 2004; Pampanin et al. 2005). Thus, individual variability in CI may mask the effect of density.

In our study, no effect of density on the slopes of the allometric Volume-Length and Projected area-Length relationships was observed until the last cultivation month (October). In this month a progressive increase was observed in the slopes of both allometric relationships with density, showing significant differences between extreme densities. However, the magnitude of the intercepts followed an inverse trend with density, whereby for the lowest densities the smallest individuals occupied a larger volume and surface area than at elevated densities, whereas the larger individuals occupied a similar volume and surface area at all tested densities. This alludes to the existence of asymmetric competition mechanisms due to the larger individuals obtaining a greater proportion of resources, thus affecting the growth of the smaller individuals (Weiner, 1990). This behaviour has been observed in other studies at elevated densities (Bertness and Grosholz 1985; Fréchette et al. 1992; Fréchette and Despland 1999; Lauzon-Guay et al. 2005b). Our findings agrees with Fréchette et al. (1992) who hypothesized that asymmetric competition reflects an increase in the slope of Body mass-Shell length allometric relationships, as opposed to symmetric competition which exclusively affects the intercept. Little information exists on the symmetric or asymmetric nature of intra-specific competition in suspended bivalves or on the underlying mechanisms of competition. Generally, asymmetric competition has been attributed to competition by physical interference between individuals, both in sessile invertebrates (Bertness and Grosholz 1985; Okamura 1986; Fréchette et al. 1992) and plants (Thomas and Weiner 1989; Weiner 1990). Symmetric competition has been more usually related to food limitation in plants (Thomas and Weiner 1989; Weiner 1990), infaunal (Peterson, 1982) and epifaunal bivalves (Fréchette and Bourget 1985a, b) not subjected to physical limitations or spatial regulation. However, the results of Fréchette and Despland (1999) and Alunno-Bruscia et al. (2001) using mussels grown in the laboratory in the absence of physical interference suggest that local seston depletion can not be discarded as cause of asymmetric competition mechanisms.

Our results corroborate the notion of density-dependent morphological plasticity as noted in previous studies (Coe 1946 -*Mytilus edulis diegensis*-; Seed 1968, 1978; Richardson and Seed 1990 and Alunno-Bruscia et al. 2001 -*Mytilus edulis*-; Tanita & Kikuchi 1957 -*Pinctada martensii*-; Ohba 1956 -*Venerupis semidecussata*-; Wade 1967 -*Donax striatus*-; Lent 1967 -*Modiolus demissus*-; Brown et al. 1976). In addition to changes in morphology related to the ontogeny of the individuals, a clear effect of density on morphological parameters has been

observed, leading to narrower and more elongated mussels at higher densities with respect to those grown at lower density. The significant interaction between density and time found in the present study reflects an increasing intra-specific competition as mussels grow, due to an increment of their space and food requirements.

Population density has been proposed to alter the morphology of mussels through competition for food (“exploitation”) or physical compression of the surrounding individuals (interference). However, due to multiple interactions between both factors, it is difficult to discriminate the principal driver of such morphological changes (Alunno-Bruscia et al. 2001; Guíñez and Castilla 1999; Guíñez 2005). Additional work is needed to clarify the degree to which each component (food limitation, food access or interference) contributes to the morphological changes of the individuals.

This work supports the hypothesis that morphological plasticity is a strategy to mitigate the effects of intra-specific competition at the individual level (Seed 1968, 1973; Brown et al. 1976). In addition, this plasticity seems to be a function of the size of individuals, leading to a greater morphological uniformity within sizes in populations cultured at lower density levels. Variations in shell morphology of mussels could have an effect on the acceptability and the value of the product in the market for fresh or frozen products, although this might not be the case for meat extraction. Our results may contribute to the adjustment of an appropriate cultivation density which would minimize intra-specific competition and optimize mussel production.

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Figure Captions

Fig. 1. Map of the Ría de Ares-Betanzos showing the topography of this inlet and highlighting the raft cultivation areas used for the fattening of mussels. Modified from Álvarez-Salgado et al. (2011).

Fig. 2. Linear and polynomial fits to the average width-length (W/L) ratios of *M. galloprovincialis* throughout the experimental culture at different densities: 220, 370 and 500 (linear); 570, 700, 800 and 1150 ind/m (polynomial).

Fig. 3. Average height-length (H/L) ratios of the different density treatments tested (N= 220, 370, 500, 570, 700, 800 and 1150 ind/m) along the experimental culture.

Fig. 4. Linear fits of the mean height-width (H/W) ratio for the different density treatments (N = 220, 370, 500, 570, 700, 800 and 1150 ind/m) along the experimental culture.

Fig. 5. Average energy distribution (DWt/DWs) of the different density treatments tested along the experimental culture.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Table 1 Mean and standard deviation of mean length ($\bar{L}$; mm), dry tissue weight (DWt; g), total dry weight (TDW; g) and morphological ratios (H/L, W/L and H/W) of mussels at the beginning of the experimental culture

$\bar{L}$	DWt	TDW	H/L	W/L	H/W
46.15±3.76	0.39±0.08	2.60±0.32	0.53±0.03	0.31±0.02	1.68±0.14

Table 2 Two way ANOVA results testing the effect of sampling time and density treatment on the different ratios analysed in the study (W/L, H/L, H/W and DWt/DWs)

Source of variation	df	S.S.	M.S.	F
W/L				
Time	5	0.074	0.015	37.72***
Density	6	0.014	0.002	5.96***
Time:Density	30	0.026	8.7e-04	2.22***
Residuals	2229	0.876	3.9e-04	
H/L				
Time	5	0.115	0.023	24.76***
Density	6	0.063	0.010	11.22***
Time:Density	30	0.046	0.002	1.65*
Residuals	2226	2.071	9.3e-04	
H/W				
Time	5	4.786	0.957	64.27***
Density	6	0.288	0.048	3.22**
Time:Density	30	0.683	0.023	1.53*
Residuals	2231	33.226	0.015	
IC				
Time	5	57,628,599	11,525,720	253.26***
Density	6	70,412	70,412	1.55
Time:Density	30	43,723	43,723	0.96
Residuals	1045	45,510	45,510	

Table 3 Parameters of the linear ($W/L = a + bx$) and second-order polynomial regression ($W/L = a + bx - cx^2$) describing the changes in the mean W/L index as a function of time

W/L	a	b	c	R ² adjusted	F	N
220	0.309	0.005**		0.737	15.03*	6
370	0.313	0.004**		0.898	45.21**	6
500	0.310	0.004**		0.917	55.96**	6
570	0.306***	0.009	-0.0008	0.683	6678.44***	6
700	0.301***	0.012**	-0.0012**	0.977	88894.48***	6
800	0.302***	0.010**	-0.0010*	0.911	45734.57***	6
1150	0.308***	0.009**	-0.0011**	0.900	68226.59***	6

* p<0.05 ** p<0.01 *** p<0.001

Table 4 Linear regression parameters describing changes in the mean H/W index as a function of time, by means of the equation: $H/W = a + bx$

H/W	Intercept	Slope	R ² adjusted	F	N
220	1.704	-0.030	0.756	16.50*	6
370	1.689	-0.029	0.955	107.77***	6
500	1.708	-0.029	0.907	49.61**	6
570	1.657	-0.022	0.838	26.95**	6
700	1.716	-0.039	0.972	175.23***	6
800	1.681	-0.026	0.969	155.22***	6
1150	1.633	-0.018	0.671	11.18*	6

* p<0.05 ** p<0.01 *** p<0.001