ORIGINAL RESEARCH

Effects of stocking density on the growth, meat yield, and survival of cultured *Mytilus platensis* in San Jorge gulf

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ABSTRACT. In Argentina, mussel farming is carried out on a very small scale in the southern Patagonian region. One of the most critical factors influencing mussel culture yield is the number of mussels seeded on socks, as this density has significant impact on growth and mortality. The objective of this study was to determine whether the stock density during the grow-out phase affects overall production performance in the farming of the mussel *Mytilus platensis*. A nine-month experimental culture socks at three densities (high: 600 mussels m^{-1}; intermediate: 450 mussels m^{-1}; and low: 300 mussels m^{-1}) was conducted in San Jorge gulf at the southwestern Atlantic Ocean. Shell length, meat biomass, and survival were measured for each treatment every three months. Mussels stocked at the intermediate density showed growth rates equivalent to those of the high- and low-density groups, but achieved significantly higher survival rates, suggesting a higher harvest yield.

Key words: Mollusk aquaculture, shellfish production.

Efectos de la densidad de siembra en el crecimiento, el rendimiento cárnico y la supervivencia de *Mytilus platensis* cultivado en el Golfo de San Jorge

RESUMEN. En Argentina, el cultivo de mejillones se realiza a muy pequeña escala en la región sur de la Patagonia. Uno de los factores más críticos que influyen en el rendimiento del cultivo es la densidad de mejillones sembrados en las redes de cultivo, ya que esta densidad tiene un impacto significativo en el crecimiento y la mortalidad. El objetivo de este estudio fue determinar si la densidad de siembra durante la fase de engorde afecta el rendimiento general de la producción en el cultivo del mejillón *Mytilus platensis*. Se realizó un cultivo experimental de nueve meses en redes de cultivo con tres densidades (alta: 600 mejillones m^{-1}; intermedia: 450 mejillones m^{-1}; y baja: 300 mejillones m^{-1}) en el Golfo San Jorge, en el Océano Atlántico Sudoccidental. Se midieron la longitud de la concha, la biomasa de carne y la supervivencia para cada tratamiento cada tres meses. Los mejillones sembrados a la densidad intermedia mostraron tasas de crecimiento equivalentes a las de los grupos de alta y baja densidad, pero alcanzaron tasas de supervivencia significativamente mayores, lo que sugiere un mayor rendimiento de la cosecha.

Palabras claves: Acuicultura de moluscos, producción de moluscos.

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INTRODUCTION

Aquaculture (including the farming of mollusks such as mussels) is expanding globally, with its economic and productive impact growing significantly in recent years. According to the FAO's 2024 report, aquaculture production has surpassed that of fishing, reaching approximately 130 million tons and providing around 22 million jobs worldwide. Mollusk farming accounts for 15.4% of total global aquaculture production, with mussel cultivation alone exceeding 1.9 million tons. Mussel farming in Argentina is done on a very modest scale in the southern Patagonian region (National Ministry of Economy 2023), and there is little knowledge regarding production rates and general culture management. Globally, mussel production is assessed using key growth metrics such as dry biomass, shell length increment, and survival (Lauzon-Guay et al. 2005; Cubillo et al. 2012, among many others). These parameters exhibit temporal variability throughout the mussel culture cycle. Since mussels are grown on socks, a major factor influencing their growth and survival is intraspecific competition for space and food. In extreme cases, this competition significantly reduces growth and survival, as some individuals are lost due to detachment (Fréchette et al. 1996, 2011; Fréchette and Bacher 1998; Alunno-Bruscia et al. 2000; Guiñez 2005; Fuentes-Santos et al. 2014). This process is called self-thinning (Fréchette and Lefraivre 1990), and is one of the main production processes to consider for adequate culture management because it is an indicator of mortality and production losses. In Spain, one of the pioneer countries in mussel cultivation, self-thinning is reduced by separating cultivation into two phases: breeding (or nursery) and grow-out. In the former, mussels grow at high stock densities until they reach an intermediate size, whereas in the latter, mussels are thinned and transferred to lower stock densities before reaching commercial size. Previous studies in Argentina (Nuevo Gulf) have established growth indices and stocking density effects for the mussel *Mytilus platensis* (Zaixso and Lizarralde 2001). Marques et al. (2020) evaluated growth and survival on breeding in San Jorge Gulf over a nine-month period employing small-sized individuals at medium and high initial densities. However, critical gaps persist concerning how stocking densities influence mussel production during the final grow-out phase (large seed stocking). This study examined how microscale density variations affected mussel production rates during the final grow-out phase in small-scale culture in San Jorge Gulf. It extends previous work by evaluating productive parameters over a nine-month period using initial shell lengths comparable to those used in some other regions during the grow out phase (Cubillo et al. 2012).

MATERIALS AND METHODS

Study site and experimental design

The study was conducted over nine months (from September 2018 to June 2019) in the central coastal area of San Jorge gulf, Argentine Patagonia (45° 59′ 12.13″ S and 67° 33′ 17.45″ W) (Figure 1). To carry out culture tests, a 100-m surface longline structure at 15 m depth was installed, considering the average tidal length.

To assess the effects of stocking density on mussel growth performance, 1-m-long culture socks were prepared following the French method (Zaixso and Lizarralde 1998). Each sock was made with an inner cotton mesh containing mussels and a 4-cm wide plastic outer mesh. Mussels were seeded at three densities: low (300 mussels m^{-1}), intermediate (450 mussels m^{-1}), and high (600 mussels m^{-1}). A total of nine socks were used for each density and placed in the sea at time t_0 in September 2018. From that moment on, three socks were sampled every three months (December 2018,

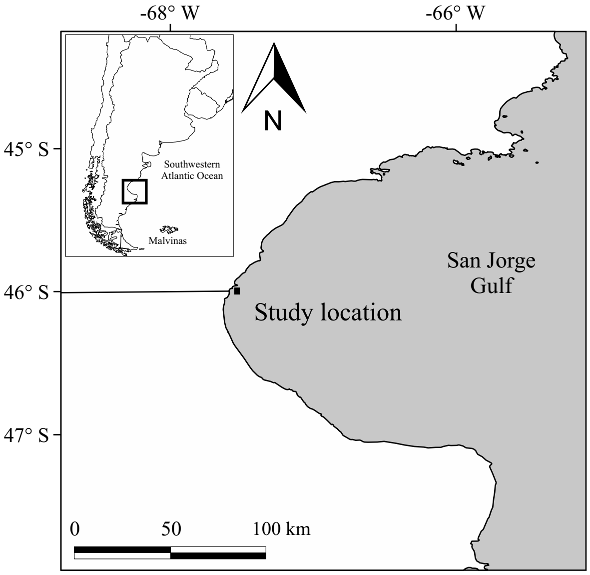

Figure 1. Study location area at San Jorge gulf, Argentine Patagonia.

March 2019, and June 2019), according to the established density completing a nine-month growth cycle. Mussels used in study were obtained from seeds developed in a hatchery. They were subsequently placed in the sea on a longline system until the time we acquired them and seeded at shell lengths of 45.72 ± 7.15 mm.

Data recording and statistical analysis

Environmental parameters, including monthly mean sea surface temperature (SST), chlorophyll-*a* concentration, and salinity, were obtained from NASA's Aqua-MODIS satellite dataset at 4-km spatial resolution.

For each initial density and sampling date, growth performance was assessed by measuring shell length and dry biomass in a random subsample of 50 mussels. Shell length was determined as the linear distance between the umbo and the anterior-posterior margin by using a digital caliper (CTM 0.01 mm). The mean monthly growth length rate (MMGLR) was calculated as the final average length minus the initial average length and divided into three monthly periods. To obtain the dry meat biomass, soft tissues were removed, dried at 65 °C for 48 h to constant weight, and then weighed with an analytical balance (OHAUS 0.0001 g). Mean shell length (MSL) and mean dry weight (MDW) were analyzed using a two-way ANCOVA model with initial shell length as a continuous covariate, and growth time (months) and initial density as fixed factors. All variables were log-transformed to meet the assumptions of normality and homoscedasticity. To identify specific treatment differences when ANCOVA revealed significant effects, non-orthogonal planned comparisons were conducted using t-tests with Šidák correction. All tests were performed using a significance level of 0.05. Due to the non-independent nature of the data, an exploratory trend analysis was performed using the average monthly growth. Finally, absolute survival (remaining mussel counts), relative survival (proportion from total seeded), and mean quarterly survival rate mean (MQSR) were quantified. Absolute and relative survival were determined by counting the number of mussels remaining in culture socks. The mean quarterly survival rate mean was calculated as remaining mean mussel counts per each density and divided by the mean initial number of mussels at the beginning of the quarterly. Survival was used to analyze the effects of stocking densities on the self-thinning process during the cultivation period. To evaluate these effects, the data (i.e. absolute and relative mussel counts) were analyzed using a two-way ANOVA ($\alpha = 0.05$), with growth time and density as fixed factors. For absolute survival data showing mean overlap, planned non-orthogonal t-tests with Šidák adjustment were performed to assess pairwise differences between density treatments. For relative survival, when two-way ANOVA indicated significant effects, Tukey's HSD *post-hoc* tests were conducted to identify specific differences among treatment groups. Due to the non-independent nature of the data, an exploratory trend analysis was performed using the average quarterly survival rate. All data were analyzed using the Statistica 7.0 program.

RESULTS

Environmental variables

During this study, SST ranged from 8.18 °C in September 2018 to 15.55 °C in February 2019, while salinity fluctuated between 32.97 in June 2018 and 33.41 in March 2019. On the other hand, chlorophyll-*a* exhibited a bimodal distribution, with peaks recorded in November 2018 (5.45 mg m^{-3}) and March 2019 (4.28 mg m^{-3}), and the lowest value observed in September (1.35 mg m^{-3}) (Figure 2).

Growth analysis

Results indicated that mussels reached local commercial size (> 75 mm) after the nine-month growth period. A general trend indicated that growth rates (MMGLR) were higher during the initial six-month growth period than during the complete nine-month growth phase (Table 1; Figure 3). The ANCOVA results confirmed a differential pattern in growth length, with a significant interaction between factors (growth time × density) [$F_{(4, 426)} = 3.64$; $p \leq 0.00$]. Nevertheless, no statistically significant differences were observed between the means (MSL) for each growth period analyzed [$F_{(2, 426)} = 0.45$; $p = 0.637$]. This trend was also evident in dry meat weight, where dry biomass gain was greater during the initial six months than in the final growth phase (Figure 4). Additionally, significant interaction between factors was identified on ANCOVA analysis (growth time × density) [$F_{(4, 426)} = 4.58$; $p \leq 0.00$]. Significant differences across biomass means for each growth period were observed only in the six-month growth period (Figure 4). T-test (with Sidak's adjustment) indicated that, at the six-month growth time, the MDW for the intermediate stocking density (450 mussels m^{-1}) was significantly lower than that for the high and low stocking densities (600 mussels m^{-1} and 300 mussels m^{-1}) with ($t_{(2, 0.016)} = -3.51213$, $\alpha \leq 0.016$) and ($t_{(2, 0.016)} = -3.23263$, $\alpha \leq 0.016$), respectively.

Survival analysis

A general analysis of mussel survival indicated that the number of mussels remaining on the cul-

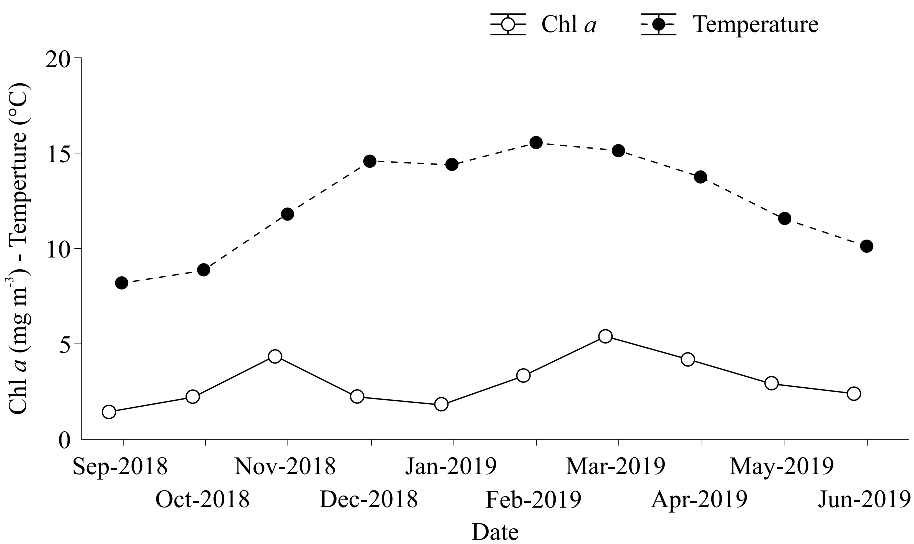

Figure 2. Monthly surface seawater temperature and chlorophyll-*a* (Chl *a*) recorded at the study site between September 2018 and June 2019.

Table 1. Mean shell length (MSL), mean monthly growth length rate (MMGLR), mean dry weight (MDW) of *Mytilus platensis* during the nine-month period. SE: standard error. N: sample number.

Growth time (month)	Density (mussels m^{-1})	MSL ± SE (mm)	MMGLR ± SE (mm $month^{-1}$)	MDW ± SE (g)	N
3	300	57.30 ± 0.70	3.57 ± 0.13	1.06 ± 0.03	50
	450	56.31 ± 0.69	3.58 ± 0.13	1.09 ± 0.04	50
	600	58.86 ± 0.62	3.52 ± 0.10	1.21 ± 0.04	50
6	300	72.35 ± 0.71	5.01 ± 0.23	1.87 ± 0.06	50
	450	70.15 ± 0.71	4.61 ± 0.23	1.61 ± 0.07	50
	600	70.27 ± 0.98	3.80 ± 0.32	1.85 ± 0.07	49
9	300	75.75 ± 0.85	1.13 ± 0.28	2.02 ± 0.07	42
	450	76.68 ± 0.92	2.17 ± 0.30	1.88 ± 0.07	48
	600	75.25 ± 0.89	1.66 ± 0.29	1.85 ± 0.08	47

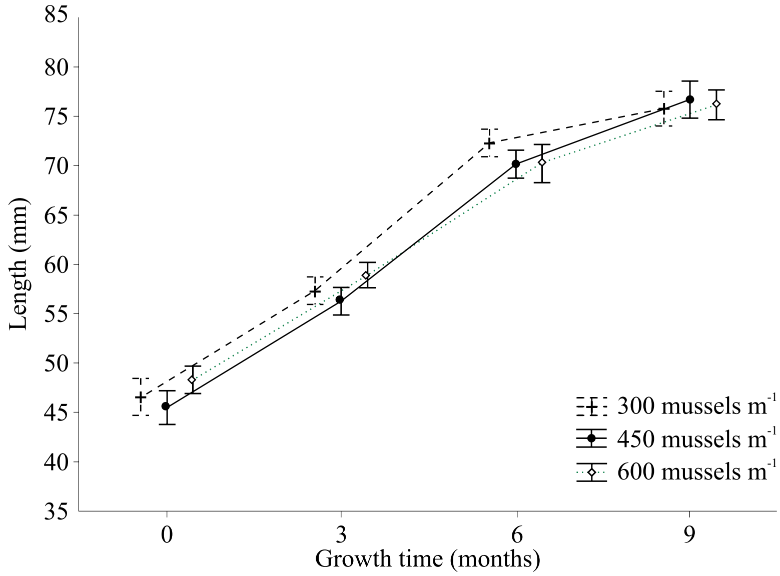

Figure 3. Length adjusted means plot. Bars denote the 95% confidence interval estimated under ANCOVA homoscedasticity assumption ($p \leq 0.05$). Initial length covariable: 45.72 mm.

ture socks decreased throughout the entire growth period. Socks seeded at lower and intermediate densities (300-450 mussels m^{-1}) exhibited a similar, steady decline. In contrast, socks seeded at higher densities showed more abrupt fluctuations in survival (Figure 5), and the survival rate between trimesters (MQSR) decreased towards the end of the study (Table 2). This differential pattern was confirmed by ANOVA results, which showed a significant interaction between factors: [$F_{(6, 18)} = 6.91$, $p < 0.05$]. Overlap in absolute survival means among density treatments was observed exclusively at the nine-month cultivation period. Šidák-adjusted pairwise comparisons at this stage

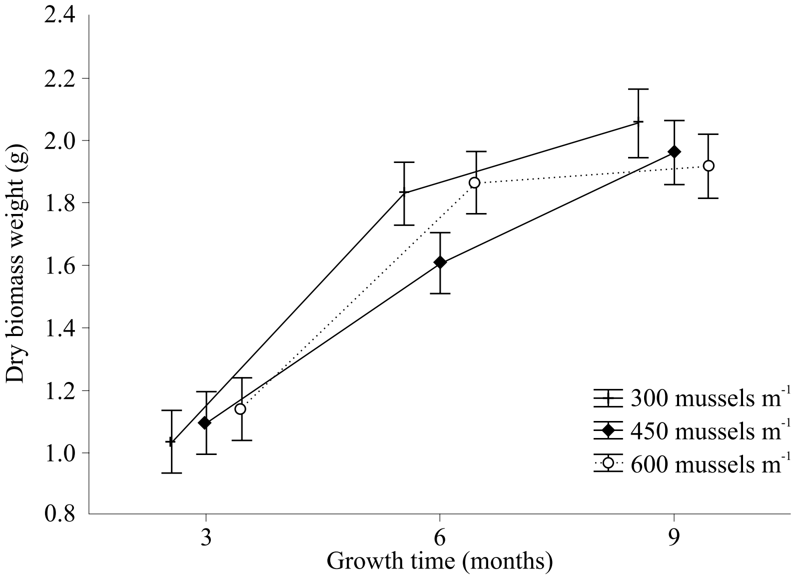

Figure 4. Dry meat biomass weight (DBW) of *Mytilus platensis* adjusted means plot. Bars denote 95% confidence interval estimated under ANCOVA homoscedasticity assumption ($p \leq 0.05$). Initial length covariable: 45.72 mm.

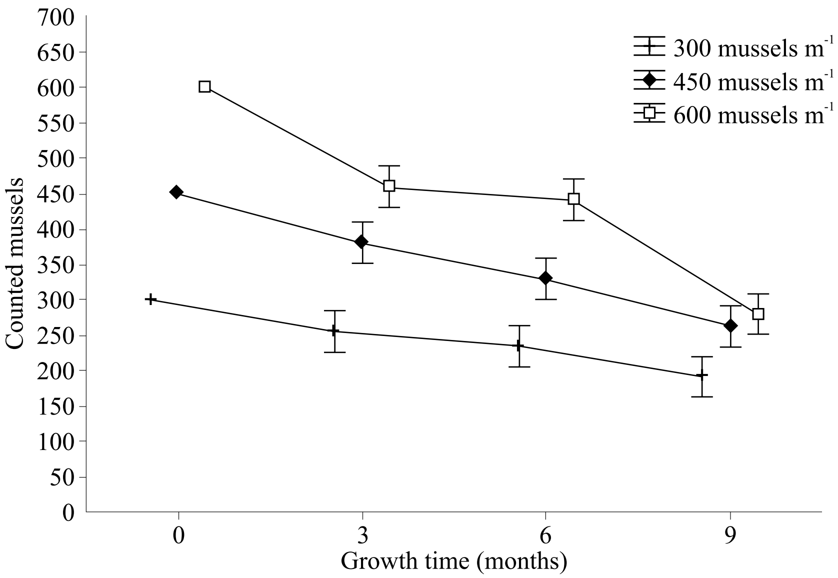

Figure 5. Absolute survival means plot of *Mytilus platensis* over a nine-month period. Bars denote 95 % confidence interval in ANOVA under homoscedasticity assumption.

demonstrated significantly lower survival at 300 mussels m^{-1} than at both 450 mussels m^{-1} ($t_{(2;\,0.016)} = 4.16$, $t_o = -3.65$, $\alpha \leq 0.016$) and 600 mussels m^{-1} ($t_{(2;\,0.016)} = 4.16$, $t_o = -4.53$, $\alpha \leq 0.016$).

The ANOVA of relative survival, which assessed differences in mean relative survival across growth periods and stocking densities, revealed statistically significant effects of both factors (growth

Table 2. Survival analysis. Mean counted mussels and relative survival (%) by density and growth time and mean quarterly survival rate (MQSR). SE: standard error.

Growth time (months)	Density (mussels m^{-1})	Mean counted mussels ± SE	Relative survival ± SE (%)	MQSR (%)
3	300	255 ± 16	85.00 ± 5.33	85.00
	450	380 ± 21	84.44 ± 4.66	84.44
	600	459 ± 43	7.50 ± 7.16	76.50
6	300	235 ± 11	78.33 ± 3.66	92.16
	450	328 ± 37	72.88 ± 8.22	86.32
	600	440 ± 27	73.33 ± 4.50	95.87
9	450	262 ± 12	58.22 ± 2.66	79.88
	600	279 ± 12	46.50 ± 2.00	63.50
	300	191 ± 5	63.66 ± 1.66	81.28

period: $F_{(2, 18)} = 66.01$, $p < 0.05$; stocking density: $F_{(2, 18)} = 9.73$, $p < 0.05$) (Figure 6). According to the Tukey HSD multiple comparison test, relative survival was density-dependent only during the final stage of the fattening period (at nine months). At this stage, the highest density (600 mussels m^{-1}) showed a significantly lower relative survival mean (46%) than the intermediate (450 mussels m^{-1}, 58%) and lowest densities (300 mussels m^{-1}, 63%) densities (Table 2).

DISCUSSION

Mussel farming is increasing globally, and one of the key research priorities is improving production yield, particularly by understanding growth and survival dynamics on culture ropes. One of the primary effects of size growth, and the consequent competition for food and space, is a reduction in the growth rate of mussels (Bayne 1976; Gosling 1992). This general trend was also observed in our study, and although it was not designed to focus on the relationship between environmental variables and production parameters, the observed decrease in growth coincided with reductions in both chlorophyll-*a* concentration and seawater temperature. This suggests that the slowdown may have been intensified by the seasonal decline in phytoplankton availability, as described in Irisarri et al. (2014), Grant et al. (2008), and Newell et al. (2004), among others. One of the most important issues on culture yield performances is related to the number of mussels seeded on socks, as this density influences growth and survival. Previous studies indicate a negative correlation between growth and stocking density (Fréchette et al. 1996, 2011; Fréchette and Bacher 1998; Alunno-Bruscia et al. 2000; Guiñez 2005; Fuentes-Santos et al. 2014; Marques et al. 2020). However, our findings suggest that during the final growth phase, stocking density had no significant effect on shell length or soft body mass. These results indicate that seeding with intermediate-sized (46 mm) mussels may reduce density-dependent effects on growth. Beyond this general trend, growth trajectories differed among stocking densities. At intermediate density, meat biomass increased in an approximately linear manner, whereas growth at low and high densities progressively approached an asymptote, consistent with the growth dynamics predicted by von Bertalanffy and Gompertz models.

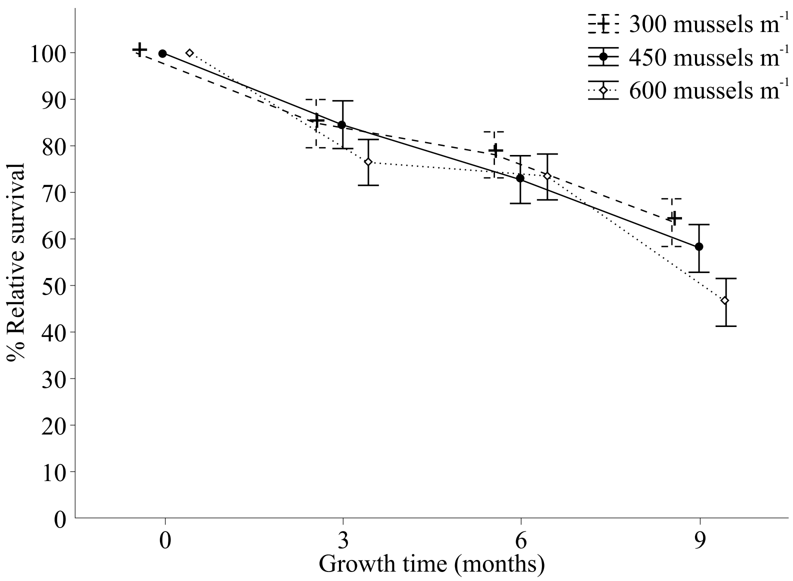

Figure 6. Plot of the mean relative survival for each growth period and stocking density of *Mytilus platensis*. Error bars represent 95% confidence intervals for the mean relative survival in ANOVA with homoscedasticity assumption.

On the other hand, mussel survival on culture socks is a time-dependent process in which intraspecific competition for space causes individuals' reduction, resulting in production losses. This phenomenon, called self-thinning process (Fréchette and Lefraivre 1990), increases with stocking density (Fréchette et al. 1996, 2011, 2014; Fréchette and Bacher 1998; Zaixso and Lizarralde 2001). Our results indicate that stocking density significantly affected survival. Although the highest density initially retained more mussels, after nine months, the number of mussels remaining at the highest and intermediate densities was similar, whereas the lowest density showed a significantly lower value. Relative survival did not differ among treatments during the first six months, but after nine months, it was significantly lower at the highest stocking density compared to the lowest and intermediate. These findings suggest that 450 mussels m^{-1} provides the best balance between survival and meat productivity, maximizing mussel retention while minimizing proportional losses. Nevertheless, these results should be considered preliminary and require validation through experiments with replicated production-scale units (considering seed provision cost) before being extrapolated to commercial farming. Overall, the combined evaluation of growth, biomass, and survival indicates that 450 mussels m^{-1} is the most suitable stocking density for the grow-out phase under the conditions evaluated. Although growth did not differ among treatments, the intermediate density achieved higher survival and, consequently, greater harvest yield. This performance is likely associated with the carrying capacity of the San Jorge Gulf, where the absence of large-scale mussel farming may reduce food limitation and seston depletion, processes commonly reported in more intensive culture areas (e.g. Zhong et al. 2024).

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Author contributions

Tomás E. Isola: conceptualization; methodology validation; formal analysis; investigation; resources; data curation, writing-original draft; writing-review and editing; visualization; supervision; project administration; funding acquisition. Francisco J. Rozzi: investigation; resources; data curation; founding acquisition. Bernardo G. Marques: investigation; data curation; writing-review and editing. Manuel García-Ulloa: writing-review and editing.

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