Revista de Biología Marina y Oceanografía
Vol. 49, Nº3: 567-575, diciembre 2014
DOI 10.4067/S0718-19572014000300013

ARTICLE

Effect of stocking density and food ration on growth and survival of veliger and pediveliger larvae of the taquilla clam *Mulinia edulis* reared in the laboratory

Efecto de la densidad de cultivo y la ración de alimento en el crecimiento y supervivencia de larvas velígeras y pedivelígeras de la almeja taquilla *Mulinia edulis* cultivadas en el laboratorio

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Resumen.- Para evaluar el efecto de la densidad sobre el crecimiento y la sobrevivencia se cultivaron larvas velígeras de la almeja taquilla *Mulinia edulis* a una densidad de 10 y 20 larvas ml^{-1}, alimentadas con 25.000 cel. ml^{-1}día^{-1} de una mezcla de *Isochrysis aff. galbana* (T-ISO) y *Nannochloropsis oculata* en recipientes de 5 L. Para medir el efecto de la ración de alimento se utilizaron 25.000 y 50.000 cel. ml^{-1}día^{-1}. Al final del experimento de densidad se encontraron diferencias en la supervivencia, pero no en el crecimiento. Las raciones de alimento experimentales no mostraron diferencias, ni en el crecimiento ni en la supervivencia larval. Para establecer el efecto de la densidad en el asentamiento, se cultivaron larvas pedivelígeras a densidades de 5, 10, 20 y 40 larvas cm^{-2}, en un sistema down-welling. Para determinar la ración adecuada se usaron 25.000, 50.000 y 100.000 cel. ml^{-1}día^{-1} de T-ISO y un cuarto tratamiento con aumento progresivo del alimento (25.000 a 100.000 cel. ml^{-1}día^{-1}). El efecto de la densidad para las larvas pedivelígeras fue significativo en el crecimiento y en la supervivencia. Las densidades más bajas presentaron mayores tamaños y supervivencia y las postlarvas alimentadas con una ración de 100.000 cel. ml^{-1}día^{-1} alcanzaron la mayor longitud valvar (1728 ± 293 µm). La mayor sobrevivencia (65%) se obtuvo con la ración ascendente a la edad de 50 días desde larva D. Para mejorar la producción masiva de semillas, se recomienda utilizar una densidad inicial de 10 larvas ml^{-1} y una concentración de microalgas de 25.000 cel. ml^{-1}día^{-1}. Para los cultivos postlarvales se recomienda una densidad de cultivo entre 5 y 10 larvas pedivelígeras cm^{-2} y una ración ascendente de alimento.

Palabras clave: Cultivo de almejas, larva D, postlarvas, Chile

Abstract.- In order to evaluate the effect of stocking density on growth and survival, veliger larvae of *Mulinia edulis* were reared at densities of 10 and 20 larvae ml^{-1} fed with a mix of 25,000 cells ml^{-1}day^{-1} of *Isochrysis aff. galbana* (T-ISO) and *Nannochloropsis oculata* in 5 L buckets. In ration experiments, larvae were fed with of 25,000 and 50,000 cells ml^{-1} day^{-1}. By the end of the stocking experiment, significant differences were found in survival but not in larval growth rate. The ration experiment showed no differences in the growth nor in the survival of the larvae. To establish the effect of stocking density on settlement of pediveliger larvae, these were reared in a down-welling system at densities of 5, 10, 20 and 40 larvae cm^{-2}. To determine an adequate food ration during settlement, pediveliger larvae were provided with 25,000, 50,000 and 100,000 cells ml^{-1}day^{-1} of T-ISO; a fourth treatment with a progressive increase of cells (25,000 to 100,000 cells ml^{-1}day^{-1}) was used. Lower density treatments showed greater sizes and survival. Postlarvae fed with a ration of 100,000 cells ml^{-1}day^{-1} had the greatest valve length (1728 ± 293 µm). The progressive treatment had the best survival (65%) at age 50 days from D larvae. To improve the productivity of larval culture for a massive seed production an initial stocking density of 10 larvae ml^{-1} and a microalgae ration of 25,000 cells ml^{-1}day^{-1} were recomended. For settlement stage we recommend a stocking density between 5 to 10 pediveliger cm^{-2} and the increasing food ration.

Key words: Clam aquaculture, D larvae, postlarvae, Chile

INTRODUCTION

Native clam culture is emerging as an alternative to traditional fishery. Among Chilean clams, the taquilla clam *Mulinia edulis* (King & Broderip, 1832) is a very apt species for artificial reproduction management for culturing, because of the presence of sexually mature specimens throughout the whole year (Oliva *et al.* 2005, Stotz *et al.* 2008, Jaramillo *et al.* 2008). Then, the presence of sexually mature specimens in most months of the year expedites the procurement of competent gametes for larval cultures. The estimated size at first sexual maturity is 31.9 mm and the mean fecundity is 8.35 x 10^6 oocytes per female (Oliva *et al.* 2005).

Also, this species is suitable to culturing because of their endurance to handling during the different rearing stages (Abarca *et al.* 2012, Oliva *et al.* 2013) and is highly marketable at about 30 mm as a 'baby' clam, although minimum harvest size regulations (5.5 cm) preclude this. Studies conducted by Stotz *et al.* (2008) in the Bay of Coquimbo demonstrated that only a small percentage of clam population exceeds the regulation size. In this way, aquaculture of small clams, has a great commercial potential.

Larval stocking density and feeding are relevant parameters for bivalve larval culture. It has been demonstrated that these parameters are closely related to growth and survivorship, as in the case of clams *Mercenaria mercenaria* (Loosanoff & Davis 1963, Castagna & Kraeuter 1981, Riisgard 1988), *Paphia malabarica* (Gireesh & Gopinathan 2008) and *Spisula solidissima* (Goldberg 1989). Food rations used for larval culture can exceed 50,000 cells $ml^{-1}day^{-1}$ (Hurley *et al.* 1997, O´Beirn *et al.* 1997). However, although high food concentrations can increase growth rates, they may also elevate mortality rates, which are explained by decreases in filtering rates and metabolism associated with high concentrations of microalgae (Sprung 1984, Gallager 1988, Doroudi *et al.* 1999). It is also important to note that the supply of microalgae represents a critical point for hatcheries or seeding centres, accounting for up to 30% of the seed production cost (Coutteau & Sorgeloos 1992).

Larval culture stocking density is inversely proportional to growth; bivalve larvae cultures with more than 20 larvae ml^{-1} increase their mortality and decrease growth rate (Hurley & Walker 1996). Helm & Bourne (2004) suggested a stocking density between 15-20 larvae ml^{-1} for *Tapes philippinarum* larvae culture and indicated that lower densities improved growth and survival.

The success of bivalve mollusc cultures, including clams, depends on the availability of seeds (Manzi & Castagna 1989). The most critical stage of the production of seeds is the settlement, between a pediveliger larvae that initiates metamorphosis and postmetamorphic growth, until postlarvae reach a size of 1 mm (Helm & Bourne 2004). Larvae are highly sensitive to environmental factors, which can affect their growth and therefore their artificial culture stages (Liu *et al.* 2006). The effects of density on clam culture are well documented (Hadley & Manzi 1984, Beal & Kraus 2002, Royo *et al.* 2002, Helm & Bourne 2004, Royo *et al.* 2005); however many of these studies were mainly focused on juveniles (valvar lengths from 3.9 mm to 10-15 mm), with very little attention to the postlarval stage. Liu *et al.* (2006) tested several stocking densities (5, 10, 20, 40 and 60 larvae ml^{-1}) for the clam *Meretrix meretrix* to study their effect on larval settling, shell size, time and survival showing an inverse relationship between rearing density and larvae final size. Oliva *et al.* (2013) determined the effect on stocking density and type of diet on the growth and survival of *M. edulis* postlarvae cultivated in sand at densities of 5, 10 and 20 postlarvae cm^{-2}. The survival was about 40% in the 5 and 10 postlarvae cm^{-2} density treatments, but the final length was significantly higher in the 5 postlarvae cm^{-2} treatment (2760 ± 293 μm). The diet which produced the greatest growth was *I. galbana* (clone T ISO).

If survivorship and growth are parameters that varies according to the stocking density and food ration levels in veliger and pediveliger larvae, then an appropriate level of these variables allow us to select the best condition for culturing. In this context, the present study aims to show some characteristics of broodstock, spawning and larval culture and to establish the effect, on an experimental scale, of different food rations and stocking densities on the growth and survival of *M. edulis* veliger larvae; and the effect of different stocking densities in a down-welling system and food rations on the growth and survival during pediveliger settlement until postlarvae > 3 mm, thus contributing to the development of a technology that allows the culturing of *M. edulis* on a commercial scale.

MATERIALS AND METHODS

BROODSTOCK, SPAWNING AND LARVAL CULTURE

Broodstock of *M. edulis* were extracted from a natural bank in the bay of Tongoy, Chile (30°18'S; 71°32'W). The

specimens were carried in an isothermal box with cold packs to the Pesquera San José S.A. hatchery, located in the same bay. To facilitate the expulsion of sand the clams were kept in filtered water for about 3 h. The broodstock were induced to release gametes through temperature changes (Vivanco *et al.* 2014). The oocytes were fertilized with a proportion of 10 spermatozoa per oocyte and incubated in a 200 L tank with a density 20 eggs ml^{-1} in 1 μm filtered and UV sterilized sea water. After 48 h, veliger larvae (D larvae) were obtained and used in the following experiments (Oliva *et al.* 2005). The age of the larva was calculated starting from the D larvae stage. The veliger larval experiments were performed with the same larval batch. The pediveliger larvae belonged to different batches produced in the same season.

Veliger larvae experiments

Effect of stocking density on veliger culture

Cultures were established with initial larval densities of 10 (control) and 20 larvae ml^{-1}, with 3 replicates per treatment. Larvae were kept in buckets with 5 L of ultraviolet-sterilized sea water filtered at 1 μm, at a temperature of 18 ± 1°C for 14 days. Continuous aeration was given along with a diet of 20,000 cells ml^{-1} day^{-1} of *Isochrysis aff. galbana* (T-ISO clone) and 5,000 cells ml^{-1} day^{-1} of *Nannochloropsis oculata*. Water was changed every second day. In order to estimate survival the larvae were sieved and concentrated in a 1 L container, homogeneously suspended by gentle agitation and a 1 ml aliquot was taken from each replicate and poured into a Sedgwick-Rafter chamber for observation in a trinocular Nikon® Eclipse E-200 microscope. From each sample, 30 larvae were selected randomly to measure shell length.

Effect of food ration on veliger cultures

M. edulis larvae were reared for 14 days in 40 L containers with ultraviolet-sterilized sea water filtered at 1 μm, at a temperature of 18 ± 1°C; and fed with 25,000 cells ml^{-1} day^{-1} (control) and 50,000 cells ml^{-1} day^{-1} of a mix of T-Iso and *N. oculata* in a proportion of 4:1 once per day. The cultures, with 3 replicates, were established with an initial density of 10 larvae ml^{-1}, with continuous aeration. Water was changed every other day, with counts, survival estimation and growth measurements as in the previous experiment.

Pediveliger larval experiments

Effect of stocking density on pediveliger culture

This experiment was performed with pediveligers larvae of 245 ± 10 μm shell length, coming from a 14 day-old culture of larvae fed with a standard food ration of 25,000 cells ml^{-1} day^{-1} at an initial stocking density of 10 larvae ml^{-1} and sea water at 18 ± 1°C. The densities tested were 5, 10 (control), 20 and 40 pediveliger larvae cm^{-2}. Pediveliger larvae were cultured in a down-welling system consisting of a 254 cm^2 sieve base (where larvae are settled) with a mesh of 177 μm (USA Standard ASTM 80), placed in a tray at a temperature of 18 ± 1°C with 25 L of seawater filtered at 1 μm and sterilized with UV light. Water flows from the tray to the sieve by air thrust through a 20 mm PVC tube. Larvae were fed with a fixed ration of 25,000 cells ml^{-1} day^{-1} once per day and the water was replaced every second day. The experiment was performed in triplicate; postlarvae were counted every sixth day for 30 days to establish their survival rates; 30 postlarvae were measured in each sample to analyse growth rates.

Effect of food ration on pediveliger culture

Experiments to evaluate the effect of food ration were performed with 15 days old pediveliger larvae with an initial length of 290 ± 10 μm. They were cultured in a static system in 1 L trays with a basal area of 160 cm^2. Trays were provided with a 1 cm layer of fine sand sieved with a 300 μm sieve and with microfiltered and UV-sterilized seawater at a temperature of 18 ± 1ºC. The water was replaced every second day.

Each tray began with around 960 larvae, which were fed with rations of 25,000 (control), 50,000 or 100,000 cells ml^{-1} day^{-1} of *Isochrysis aff. galbana* (clon T-ISO); a fourth trial began with 25,000 cells ml^{-1} day^{-1} and was increased by 25,000 cells every 7 days up to 100,000 cells ml^{-1} day^{-1} once per day. Each treatment was replicated four times; growth were recorded every 7 days and survival at the end of the experiment (at age 50 days from D larvae).

Statistical analysis

The Shapiro test was used to evaluate the normality of the growth data (shell lengths). The effect of stocking density and food rations on growth of larval cultures was analysed using the Kruskal-Wallis non-parametric test. The same test was used for the evaluation of stocking density on the growth of the postlarval cultures; while

for *a posteriori* analyses the Mann-Whitney U test was used (Sokal & Rohlf 2012). Survival data were arcsine transformed for further analysis. To evaluate the effect of food ration and stocking density on larval and postlarval survival, an ANOVA was used along with the *a posteriori* Fisher test (Sokal & Rohlf 2012). $P < 0.05$ was used as a significance criterion for all tests.

Results

Broodstock, spawning and larval culture

Mulinia edulis has separate sexes and no external sexual dimorphism. The female gonads have a dark purple colour, while male gonads have an orange-yellow colouring. The gametes, of whitish colour in males and red colour in females, are expelled to the environment.

The oocytes obtained after the induction of gamete expulsion are spherical and of red colour, with a mean diameter of 58 ± 1.9 μm. The average fecundation rate was 74 ± 10%. After 48 h from fecundation, the resulting veliger larvae have a 'D' shape with a straight hinge, and measure an average of 93.7 ± 5.4 μm. They present a ciliated structure, the *velum*, which allows the larvae to swim freely and catch food. Five days later the umbo of the larvae is conspicuous (Fig. 1).

After 3 to 5 days, the first umbonate larvae were found with a contractile organ covered with cilia, corresponding to the foot. This occurred at a mean shell length of 245 μm. From 2 to 4 days later, the entire batch presented a foot (Fig. 1). The pediveliger swimming larva transforms into a benthic organism when it sheds its velum at age 22 days and 0.5 mm length. This clam pediveliger larva does not present an eyespot. The metamorphosed larva has all the morphological characteristics of an adult when 28 days old and hereinafter is called postlarvae.

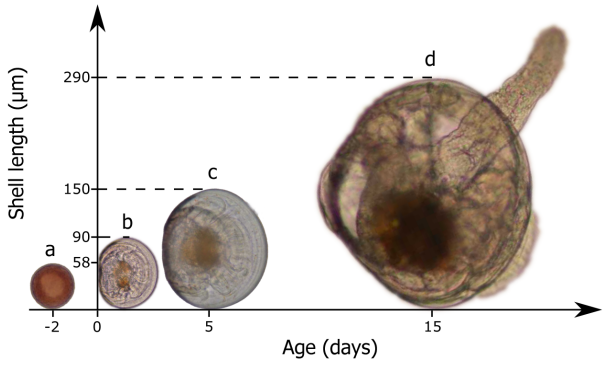

Figure 1. Larval development of the taquilla clam *Mulinia edulis*. (a) fecundated oocyte (58 μm), (b) 48 h old veliger larvae (D larvae) (94 μm), (c) 5 day old umbonated veliger (150 μm), (d) 15 day old pediveliger (290 μm) / Desarrollo larvario de la almeja taquilla *Mulinia edulis*. (a) ovocito fecundado (58 μm), (b) larvas veliger de 48 h de edad (larvas D) (94 μm), (c) larva velígera umbonada de 5 días de edad (150 μm), (d) larva pedivelígera de 15 días de edad (290 μm)

Veliger larvae experiments

Effect of stocking density in veliger culture

After 14 days of culture (starting from D larvae) *M. edulis* larvae reached shell lengths of 223 ± 22.9 μm and 216 ± 21.2 μm in cultures with initial densities of 10 and 20 larvae ml^{-1}, respectively. No significant length difference between densities was found throughout almost the entire experiment (Fig. 2A), with the exception of day 8. The larval daily growth rate during the culture period was 9.2 μm day^{-1} for the lower density (10 larvae ml^{-1}) cultures and 8.8 μm day^{-1} for cultures of 20 larvae ml^{-1}.

Larval survival was significantly greater at 10 larvae ml^{-1} (43.9 ± 4.4%) than at 20 larvae ml^{-1} (24.1 ± 0.9%) (Fig. 2B). At the end of the experiment both treatments had a similar larval density; 4.82 for the 10 larvae ml^{-1} treatment and 4.4 for 20 larvae ml^{-1}.

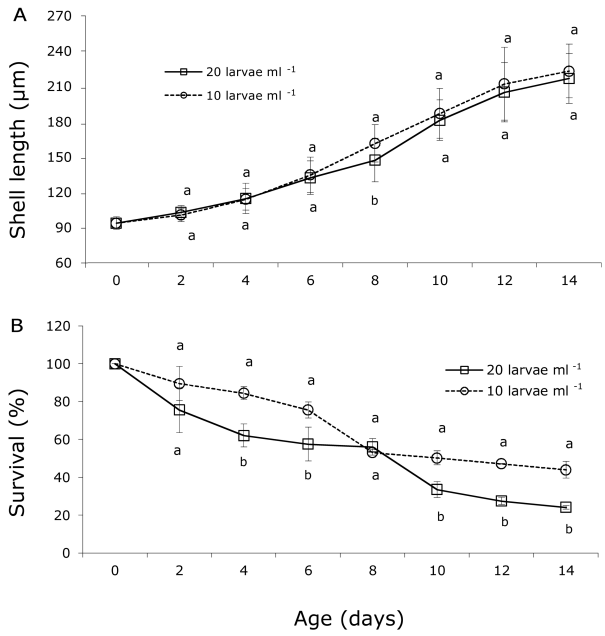

Figure 2. Growth (A) and survival (B) of *Mulinia edulis* larvae reared at two experimental densities: 10 and 20 larvae ml^{-1}. Means with different letters are significantly different ($P < 0.05$) / Crecimiento (A) y supervivencia (B) de larvas de *Mulinia edulis* cultivadas en dos densidades experimentales: 10 y 20 larvas ml^{-1}. Los promedios con letras diferentes son significativamente diferentes ($P < 0,05$)

Effect of food ration on veliger culture

Larvae fed with a daily ration of 25,000 cells ml^{-1} day^{-1} reached a mean length of 225.9 ± 23 mm by the 14th day of culture, while those fed with 50,000 cells ml^{-1} day^{-1} reached a length of 229 ± 20 mm (Fig. 3A); the difference was not significant. Daily growth rate was 8.07 and 8.27 mm day^{-1} for larvae fed with 25,000 and 50,000 cells ml^{-1} day^{-1}, respectively.

The survivorship was similar for both treatments; 34.4 ± 12% for cultures fed with 25,000 cells ml^{-1} day^{-1} and 34.9 ± 7% for cultures fed with 50,000 cells ml^{-1} day^{-1} (Fig. 3B).

Pediveliger larvae experiments

Effect of stocking density on pediveliger culture

Around age 26 days the metamorphosis occurred, and from that until the end of the experiment, postlarval growth was significantly affected by density. The treatment with 5 pediveliger cm^{-2} presented the greatest shell lengths from age 26 days onwards throughout the entire study period (2370 ± 554 μm) and the mean daily growth rate was 71 μm day^{-1}. (Fig. 4A). Postlarvae kept at an initial stocking density of 10 pediveliger cm^{-2} presented a greater growth (1733 ± 331 μm) than those kept at an initial density of 20 (1309 ± 276 μm) and 40 (1260 ± 276 μm) pediveliger cm^{-2} ($P < 0.05$).

At age 20 days, survival fell sharply to 49.8 ± 15% in the 40 pediveliger cm^{-2} treatment (Fig. 4B), which stood as the lowest of the treatments. Survival decreased to 80% in the other treatments, with no significant difference between them. There was no significant difference between treatments at age 26 and 32 days; however, survival was affected by density at age 38 and 44 days. The 40 pediveliger cm^{-2} treatment had the lowest survival; 32 ± 7% at the end of the experiment. Survival in the 5 and 10 pediveliger cm^{-2} treatments presented no significant differences during the entire experiment, with percentages of 68 ± 9% and 64 ± 22%, respectively at the end of the experiment. The 20 pediveliger cm^{-2} treatment was suspended at age 32 days, because the replicates were contaminated with protozoa.

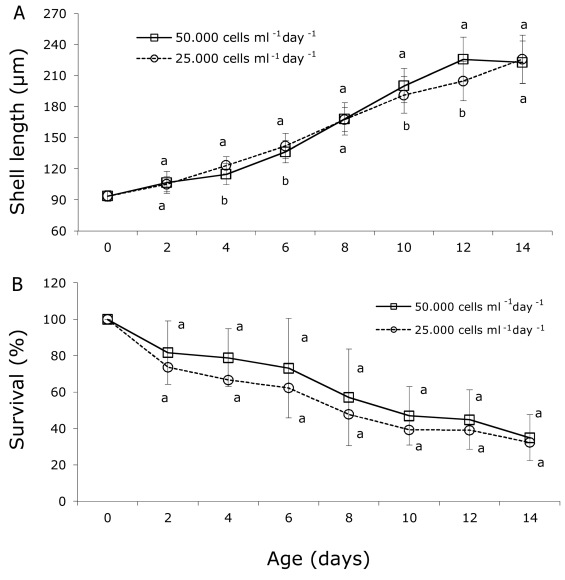

Figure 3. Growth (A) and survival (B) of *Mulinia edulis* larvae reared with two experimental food rations: 25,000 and 50,000 cells ml^{-1} day^{-1} of microalgae per day. Means with different letters are significantly different ($P < 0.05$) / Crecimiento (A) y supervivencia (B) de larvas de *Mulinia edulis* cultivadas con dos raciones de alimentos experimentales: 25.000 y 50.000 cel. ml^{-1} día $^{-1}$ de microalgas por día. Los promedios con letras diferentes son significativamente diferentes ($P < 0,05$)

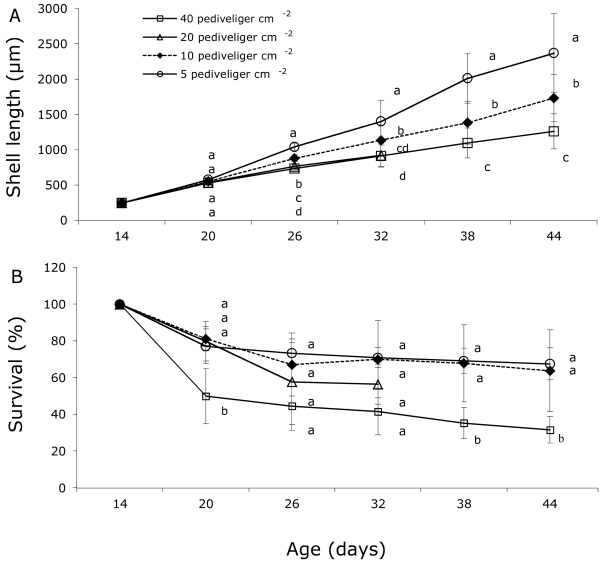

Figure 4. Growth (A) and survival (B) of *Mulinia edulis* postlarvae reared in a down-welling system with 4 densities (5, 10, 20 and 40 pediveliger cm^{-2}). Means with different letters are significantly different ($P < 0.05$) / Crecimiento (A) y supervivencia (B) de postlarvas de *Mulinia edulis* cultivadas en un sistema down-welling con 4 densidades (5, 10, 20 y 40 pediveliger cm^{-2}). Los promedios con letras diferentes son significativamente diferentes ($P < 0,05$)

Effect of food ration on pediveliger culture

The shell length of postlarvae was significantly affected by food concentration (Fig. 5). Those fed with 25,000 or 50,000 cells ml^{-1} day^{-1} grew slower than those fed with 100,000 cells ml^{-1} day^{-1}, and the difference was already significant at day 14. Those fed with the progressive increase of cells fell behind the 100,000 cell treatment in the first two weeks (when they received less food), and never quite caught up to the 100,000 cell treatment, although they maintained a similar growth rate once they received the maximum food. Interestingly, all treatments grew at about the same rate until age 22 days; the differences were established during the second week of the experiment. The overall daily growth rates of the postlarvae for the five weeks of the experiment were 25 μm day^{-1} for the 25,000 cells ml^{-1} day^{-1} ration, 31 μm day^{-1} for the 50,000 cells ration, 41 μm day^{-1} for the 100,000 cells ration, and 38 μm day^{-1} for the progressive treatment. The maximum and minimum mean shell lengths at the end of the experiment were 1728 ± 293 and 1153 ± 214 μm for the 100,000 and 25,000 cells ml^{-1} day^{-1} rations, respectively; all four treatment means were significantly different at the end of the experiment (Fig. 5).

Figure 6 shows the mean survival for the different food rations. The best survival (65.5%) was in the progressive ration treatment, and the poorest was in the postlarvae fed with 25,000 cells ml^{-1} day^{-1} (49.1%).

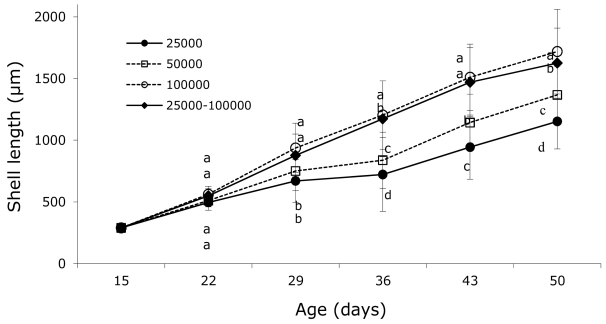

Figure 5. Growth of *Mulinia edulis* postlarvae reared in a closed system with sand with 4 different food rations (25,000, 50,000, 100,000 cells ml^{-1} day^{-1} and 25,000-100,000 cells ml^{-1} day^{-1}). Means with different letters were significantly different ($P < 0.05$) / Crecimiento de postlarvas de *Mulinia edulis* cultivadas en un sistema cerrado con arena y con 4 raciones de alimentos diferentes (25.000, 50.000, 100.000 cel. ml^{-1} $día^{-1}$ y 25.000-100.000 cel. ml^{-1} $día^{-1}$). Promedios con letras diferentes son significativamente diferentes ($P < 0,05$)

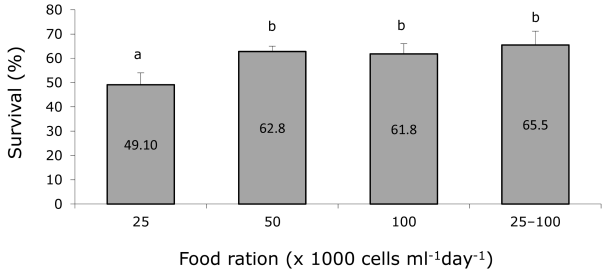

Figure 6. Survival of 50 days old *Mulinia edulis* postlarvae reared in a closed system with sand with 4 different food rations (25,000, 50,000, 100,000 cells ml^{-1} day^{-1} and 25,000-100,000 cells ml^{-1} day^{-1}). Means with different letters were significantly different ($P < 0.05$) / Supervivencia de postlarvas de *Mulinia edulis* de 50 días de edad, cultivadas en un sistema cerrado con arena y con 4 raciones de alimentos diferentes (25.000, 50.000, 100.000 cel. ml^{-1} $día^{-1}$ y 25.000-100.000 cel. ml^{-1} $día^{-1}$). Promedios con letras diferentes son significativamente diferentes ($P < 0,05$)

Discussion

The best results in terms of growth and survival in larval cultures for *M. edulis* were obtained with control density, 10 larvae ml^{-1}. Although no significant difference was found between the growth of the 10 and 20 larvae ml^{-1} treatments, the culture that began with a density of 10 larvae ml^{-1} showed significantly greater survival. Hurley & Walker (1996) found no significant difference rearing cultures of *Spisula solidissima similis* at densities of 10, 20, 30 and 50 larvae ml^{-1}, but found a significant difference for growth, which was greater for larvae cultured at the lower density of 10 larvae ml^{-1}. These authors proposed that the greater growth at 10 larvae ml^{-1} could be due to the fact that at this density larvae have a greater availability of food than larvae kept at greater densities. This may have been the case for *M. edulis*, since the food ration was four times smaller (25,000 cells ml^{-1} day^{-1}) than that used by Hurley & Walker (1996). On the other hand, Yan *et al.* (2006), working with the japanese clam *Ruditapes philippinarum*, found no difference in growth between densities of 10, 15 and 20 larvae ml^{-1}. Larval cultures of the clam *Meretrix meretrix* (Liu *et al.* 2006) at densities of 5, 10, 20, 40 and 60 larvae ml^{-1} presented the same tendency, showing no difference for densities of 10, 20 and 40 larvae ml^{-1}, and a difference for 5 larvae ml^{-1}, which presented a greater growth. Liu *et al.* (2006) recommend using moderate densities of 10 to 20 larvae ml^{-1} for massive hatchery production, because they allow rapid growth, short settling times and high survival rates.

The decrease in survival of cultures with an initial density of 20 larvae ml^{-1} may be explained according to the proposition of Liu *et al.* (2006), which attributes high mortality in larviculture to poor water quality or diseases. As the stocking density increases, more metabolic waste matter is accumulated in the water, which produces a decrease in its quality and also if the ration is equal for different larval densities than the availability of food is inversely to the stocking density. Larvae of *M. edulis* showed no significant difference larval growth or survivorship between the daily rations of 25,000 and 50,000 cells ml^{-1} day^{-1}. This suggests that 25,000 cells ml^{-1} day^{-1} may be sufficient for the larval culture stage and the daily growth rate obtained was similar to the growth reported by other authors who used greater rations such as Hurley & Walker (1996), who fed *Spisula. solidissima similis* larvae with rations of 100,000 cells ml^{-1} day^{-1}; and O'Beirn *et al.* (1997), with rations of 50,000 and 75,000 cells ml^{-1} day^{-1} for larvae of *S. solidissima solidissima*.

High microalgae rations affect the survivorship of cultures, because those cells that are not eaten by larvae suffer microbial decomposition processes, exposing cultures to noxious bacteria and fungi (Loosanoff & Davis 1963, Helm & Bourne 2004, Liu *et al* 2006). This was corroborated by Hurley *et al.* (1997), who concluded that rations greater than 200,000 cells ml^{-1} day^{-1} are excessive and are less productive, finding that the optimal for good growth and survival of *S. solidissima similis* larvae is a ration somewhere between 50,000 and 100,000 cells ml^{-1} day^{-1}.

The greater food availability for larvae fed with 50,000 cells ml^{-1} day^{-1} showed no positive effect on the growth and/or survivorship of larvae of *M. edulis*, however, the effect of a better nutrition could express itself in the metamorphosis stage, either by increasing survivorship or by shortening the stage (Marshall *et al* 2010).

The veliger larvae were fed with a mixed diet of *Isochrysis* aff. *galbana* (T-ISO clone) and *Nannochloropsis oculata*. Vivanco *et al.* (2014) assessed the effect of nine different diets on growth and survival of *M. edulis* veliger larvae. The best results (shell length= 272 μm; growth rate = 9.2 μm day^{-1}) were obtained with that a mixed diet including *Isochrysis aff. galbana* (T-ISO clone). Nevertheless, in post-larvae the suggested diet is a unialgal T-ISO clone diet (Oliva *et al.* 2013).

The settling stage, between the appearance of the foot and the loss of the velum, has no diagnostic character in *M. edulis*, like in other clam species. *Tapes* clams show no evidence of an eyespot (Jones *et al.* 1993), nor do Chilean clam larvae of *Venus antiqua* and *Gari solida* (Olavarría *et al.* 1996).

The size of postlarvae stocked at 5 pediveliger cm^{-2} was 28% greater than that of postlarvae stocked at 10 pediveliger cm^{-2}. According to Liu *et al.* (2006), stocking density affects not only larval development rate, but also the size (shell length) that it reaches once the pelagic phase ends, as we found for *M. edulis*.

M. edulis begins the pediveliger stage with the appearance of the foot, at a medium shell length of 245 μm. This happens between days 14 and 16 at a temperature of 18°C (this study) and between days 18 and 20 at 15°C (Vivanco *et al.* 2014). For *Venus antiqua* and *Gari solida*, two other commercial chilean clams, the pediveliger size is 310 μm and 279 μm at 25 and 31 days of culture respectively (Olavarría *et al.* 1996) with temperatures between 16 and 18°C (Bustos & Olavarría 2000). As mentioned previously, in the larval stage at higher densities in cultivation, metabolic waste matter could affect larval growth. The same effect may occur during postlarval growth. On the other hand, competition for space and food could explain the greater growth reached by postlarvae kept at lower densities (Yan *et al.* 2006).

Interspecies discrepancies make comparison between different clam species difficult. One example of such differences is the case of *Cyclina sinensis* (Liu *et al.* 2002), which has a much shorter larval time and smaller size at metamorphosis than *M. edulis*, which allows *C. sinensis* to endure higher stocking densities in the postlarval stage (723 postlarvae cm^{-2}).

Survivorship was affected by density; the best results were obtained in postlarvae stocked at 5 and 10 pediveliger cm^{-2}, with 68 and 64% survivorship, respectively. This survivorship is greater than that reported by Liu *et al.* 2002, which was 57.6%, however, they ended with a density of 723 postlarvae cm^{-2} (259 μm) *versus M. edulis*, which ended with a density of 7 postlarvae cm^{-2} (2377 μm).

For *M. edulis*, the stocking density used during the postlarval stage is an important factor for growth and survivorship, unlike the larval stage. Therefore this species requires special conditions for each developmental stage to obtain greater culture efficiency. By comparing the pediveliger larvae stocking density experiments in a close system with sand (Oliva *et al.* 2013) with a down-welling system (at 18 ± 1°C), in both cases the best results were obtained in the 5 and 10 pediveliger cm^{-2} treatment. But the survival is much higher in the down-welling system in 5 and 10 pediveliger cm^{-2} treatment (68 and 64%) than in the close system with sand (38 and 40%).

Different food rations had a significant effect on the increase of valve length of postlarvae of *M. edulis*. In this stage of growth, a fixed ration of 100,000 cells ml^{-1} day^{-1} and

the increasing treatment were optimal for good growth in a closed culture system with a sandy bottom.

The optimization of food availability can maximize larval growth and minimize hatchery costs (Doroudi & Southgate 2000). Knowing the optimum food concentration for postlarvae allows us to be efficient in hatchery culture costs, since an insufficient food concentration produces sub-optimal growth and overfeeding increases hatchery costs and may reduce the water quality, which may affect postlarval growth and survival. Excessive food concentrations may interfere with the food capture mechanisms of these bivalves; some of the filtered particles may be eliminated as pseudofeces, thus reducing the rate of ingestion (Albentosa *et al.* 1996). Also, the reduction in filtration rate due to high concentrations of microalgae was associated with the maximum retention time in the intestine, valve closing, reduction of metabolism and reduction in biosynthesis, and thus growth (Riisgard 1991).

Ingestion rate and absorption efficiency are physiological processes which affect the growth of seeds. In the case of the clam *Ruditapes decussatus*, the rate of ingestion increased with food concentration, but only up to a maximum of 100,000 cells ml^{-1} day^{-1}; a greater concentration produced a decrease in ingestion (Albentosa *et al.* 1996).

Food concentration had no effect on the survival of the postlarvae of *M. edulis*; except for the 25,000 cells ml^{-1} day^{-1} treatment. There were no significant differences at any of the three higher concentrations used. We obtained a mean survival between 62 and 65% with concentrations of 50,000, 100,000 and 25,000 to 100,000 cells ml^{-1} day^{-1}.

This study determined that in order to improve the mass culture of *M. edulis*, an initial stocking density of 10 larvae ml^{-1} and a daily food ration of 25,000 cells ml^{-1} of microalgae should be used for the larval stage. During the postlarval stage, the density that achieves an appropriate growth rate is 5 postlarvae cm^{-2} with an increasing food ration (25,000 to 100,000 cells ml^{-1} day^{-1}) in a down-welling system. However for commercial purposes the settlement density should be close to 10 postlarvae cm^{-2}.

Some challenges and unanswered questions are whether the parameters obtained for the larvae and postlarvae culture are static throughout the year and whether the technology developed is applicable to clam stocks from the southernmost latitudes. It is also necessary to couple the parameter estimations obtained with production costs and time of permanence in the hatchery and nursery to obtain of seeds for massive grow out.

Acknowledgments

The authors wish to thank Humberto Monsalve and Rafael Sepúlveda, of Pesquera San José for providing facilities for the experiments and the technical team of the Hatchery for support and helpful comments; Lafayette Eaton for improving the manuscript. Finally we also thank FONDEF for their support to the development of the technology for culturing native species (Project D98I1081, AQ08I1027).

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Received 11 July 2014 and accepted 27 October 2014

Associated Editor: Maritza Sepúlveda M.