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Article

Effect of stocking density and diet on growth and survival of post-larvae of the taquilla clam *Mulinia edulis* cultivated in sand in a hatchery

Efecto de la densidad de cultivo y dieta sobre el crecimiento y supervivencia de postlarvas de la almeja taquilla *Mulinia edulis* cultivadas en arena en un hatchery

Doris Oliva[1], Alejandro Abarca[1,2], Rodrigo Gutiérrez[1], Ángela Celis[1], Lucía Herrera[1] and Vanessa Pizarro

[1]Departamento de Biología & Ciencias Ambientales, Facultad de Ciencias, Universidad de Valparaíso, Centro de Investigación y Gestión de los Recursos Naturales (CIGREN), Gran Bretaña #1111, Valparaíso, Chile. doris.oliva@uv.cl
[2]Programa de Magister en Acuicultura, Facultad de Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile

Resumen.- Con el objeto de contribuir al desarrollo de la tecnología para el cultivo comercial de la almeja taquilla *Mulinia edulis,* se determinó el efecto de la densidad de cultivo y el tipo de dieta en el crecimiento y sobrevivencia de las postlarvas. Cultivos larvales de 14 días, dieron origen a postlarvas que fueron cultivadas en sistemas cerrados con fondo de arena por 30 días, a densidades de 5, 10 y 20 postlarvas cm^{-2}. Para determinar el efecto del tipo de dieta se ofrecieron 3 dietas monoespecíficas: *Isochrysis aff galbana* (clon T-ISO), *Phaeodactylum tricornotum* y *Tetraselmis suecica* y 2 dietas mixtas: *I. galbana* (clon T-ISO) (50%) – *P. tricornotum* (50%) y *I. galbana* (clon T-ISO) (33%) – *P. tricornotum* (33%) – *T. suecica* (33%) a una ración fija de 100.000 cel ml^{-1} $día^{-1}$. El cultivo a una densidad 5 postlarvas cm^{-2} generó la mayor longitud valvar que fue de 1707 ± 200 µm a una edad de 44 días. Las postlarvas alimentadas con *Isochrysis galbana* (clon T-ISO) alcanzaron las mayores longitudes valvares, 2225 ± 430 µm a una edad de 50 días. La supervivencia de las postlarvas fue alrededor del 50% y no fue afectada por los diferentes niveles de parámetros analizados (densidad de cultivo y dieta).

Palabras clave: Cultivo de almejas, Mactridae, hatchery

Abstract.- We determined the effect of stocking density and type of diet on the growth and survival of post-larvae of the taquilla clam *Mulinia edulis*, as a contribution to the development of technology for the commercial cultivation of this species. Larval cultures of 14 days gave rise to postlarvae which were cultivated in closed systems in sand for 30 days, at densities of 5, 10 and 20 post-larvae cm^{-2}. To determine the effect of diet, 3 monospecific diets (*Isochrysis aff galbana* (clone T-ISO), *Phaeodactylum tricornotum* and *Tetraselmis suecica*) and 2 mixed diets [*I. galbana* (clon T-ISO) (50%) – *P. tricornotum* (50%) and *I. galbana* (clon T-ISO) (33%) – *P. tricornotum* (33%) – *T. suecica* (33%)] were provided at a fixed total ration of 100,000 cell ml^{-1} day^{-1}. Cultivation at a density of 5 post-larvae cm^{-2} produced the greatest valve length at age 44 days, 1707 ± 200 µm. Postlarvae fed with *Isochrysis galbana* (clone T-ISO) had the greatest valve length, 2225 ± 430 µm at age 50 days. The survival of postlarvae was about 50%, and was not affected by the different levels of the parameters analyzed (cultivation density and diet).

Key words: Clam culture, Mactridae, hatchery

Introduction

Mulinia edulis (King & Broderip, 1832), commonly called the 'taquilla' clam, is one of 6 species of clam commercially exploited in Chile (Abarca *et al.* 2012). Extraction is restricted to individuals with a valve length of at least 55 mm and is only performed by local fishermen from historical fishery grounds and management areas of benthic resources (Oliva *et al.* 2005). The decrease in landings recorded in Chile in the last decade of the different species of clams (SERNAPESCA 2010) has generated problems in the supply of raw material for the processing plants which export these products, mainly to the European Community and countries of the Asian Pacific (Oliva *et al*. 2005).

Among the Chilean clams, *M. edulis* is a dioecious species especially apt for the artificial management of reproduction for cultivation, due to the presence of sexually mature individuals during the entire year, which

permits to obtain competent gametes for larval cultures (Oliva *et al.* 2005).

The settling process initiated with the appearance of a contractile organ covered with cilia, the foot. This occurred at a mean valve length of 200 µm (14 days old). In contrast to other bivalves, the postlarvae of *Mulinia edulis* do not have an eye spot. The swimming pediveliger transforms into a benthic organism by eliminating the velum after about 22 days. The metamorphosed larva has all the morphological characteristics of an adult at 28 days, when it has a valve length of 1 mm. The shell continues to have a translucent white color, which allows all its internal organs to be seen (Oliva *et al.* 2005).

According to Wang *et al*. (2008) a large scale clam culture industry depends on seed production. Therefore, nutrition in larval stages plays a key role in successful seed rearing. Temperature and food availability are the principal factors which affect the growth rate of clam larvae (Albentosa *et al.* 1996a, Liu *et al.* 2006). Diet requirements are specific for different clam species (Albentosa 1996b, Helm & Laing 1987), therefore it is critical for successful culture to select an adequate diet (Tang *et al.* 2006).

A number of studies have evaluated diets for the larval stage of different species of clams: *Mercenaria mercenaria*, *Tapes semidecussata* (Helm & Laing 1987), *Meretrix meretrix* (Tang *et al*. 2006), *Ruditapes philippinarum* (Yan *et al.* 2006), *Paphia malabarica* (Raghavan & Gopinathan 2008), as well as for other bivalve species such as the oyster *Pinctada margaritifera* (Southgate *et al.* 1998, Martínez-Fernández *et al.* 2006) and the scallop *Argopecten ventricosus-circularis* (Lora-Vilchis & Maeda-Martínez 1997).

Cultivation density is an important factor which affects not only the rate of larval development, but also the size at the end of the pelagic phase, settling and metamorphosis. Taylor *et al.* (1998), in larval cultures of the silver-lip pearl oyster *Pinctada maxima*, and Liu *et al.* (2006), with the clam *Meretrix meretrix*, showed that larval density affects both the size of the larvae and the time they take to reach the stage of metamorphosis.

The great majority of the density studies performed with filtering mollusks have used volumetric rather than area densities in the postlarval stage (Taylor *et al.* 1998, Doroudi & Southgate 2000). Density studies (individuals per cm^2 or m^2) have focused on seeds and individuals longer than 3 mm (Hadley & Manzi 1984, Doménech 1994, Liu *et al.* 2002, Royo *et al.* 2005 a, b).

To develop the culture technology of *M. edulis* it is important to know the optimum diet and cultivation density in postlarvae, in order to scale them appropriately. There is currently a lack of information on the influence of diet and stocking density on the development of *M. edulis* postlarvae. We hypothesized that low stocking density will result in a better growth rate and better survival compared with higher stocking densities. We also expect that mixed diets will perform better than monospecific diets in the growth and survival of postlarvae. Therefore, the aim of this study is to evaluate the effects of these factors on the growth and survival of *M. edulis* postlarvae cultivated in sand.

Materials and Methods

The experimental culture of postlarvae of taquilla clam was performed in the hatchery of the Pesquera San José S.A, located in the Tongoy Bay (30º15'27''S; 71º29'33''W), Coquimbo, Chile, from September, 2002 to June, 2003.

Broodstock were extracted from a natural bank located near Puerto Aldea (30º17'31''S; 71º36'32''W) in Tongoy Bay. The postlarvae (hereafter PL) were obtained from 14-day larval cultures with an initial density of 10 larvae ml^{-1}, fed with a ration of 25.000 cell ml^{-1} day^{-1} and cultivated in sea water filtered at 1µm and sterilized with UV light, at a temperature of 18 ± 1ºC. For postlarval settlement sand was used as substrate and was first sifted in a 300 µm sieve and sterilized at 100ºC for 5 min. The cultures were controlled every 6 or 8 days. On every occasion, the sand was sifted in a 300 µm sieve (USA standard ASTM N°50). The PL retained in the sieve were counted to determine survival, and the shell length was measured in 30 individuals selected at random to analyze growth. The sea water was changed every 2 days and the cultures were maintained with continuous aeration and with a photoperiod of 12 h light and 12 h dark in containers of 160 cm^2.

Effect of stocking density on postlarval culture

PL of *M. edulis* with an initial length 265 ± 12 µm were maintained for 30 days with sea water filtered at 1 µm and sterilized with UV light at a temperature of 17 ± 1ºC. They were fed with a mixture of *Isochrysis galbana* (90%) and *Phaeodactylum tricornutum* (10%). The food ration was 25,000 cell ml^{-1} day^{-1} for the first 7 days, 50,000 cell ml^{-1} day^{-1} in the second week, 100,000 cell ml^{-1} day^{-1} in the third week and 200,000 cell ml^{-1} day^{-1} until the end of the experiment.

The experiments of stocking density were performed in 50 L wooden trays covered with epoxy resin with a basal area of 0.5 m^2 which were covered with 2 cm of sand. We tested 3 stocking densities, with 5, 10 and 20 postlarvae cm^{-2} (PL cm^{-2}). The treatments were replicated 3 times; growth was evaluated every 8 days. Survival was evaluated at days 36 and 44, when the postlarvae had reached a size greater than the sieve mesh (300 µm) and thus were retained on sifting.

Effect of diet on postlarval culture

The diet experiments were conducted with postlarvae which began with a valve length of 271 ± 13 µm, from 19-day larval cultures. The PL were cultivated in 1 L plastic trays with a basal area of 160 cm^2 covered with 1 cm of sand, in micro-filtered sea water sterilized with UV light, at a temperature of 18ºC. The initial density was 6.54 PL cm^{-2}. The treatments consisted of 3 monospecific diets of microalgae: *Isochrysis aff. galbana* (clone T-ISO) (*I*), *Phaeodactylum tricornutum* (*P*) and *Tetraselmis suecica* (*T*), and 2 mixed diets: *I. galbana* (clone T-ISO) (50%) – *P. tricornutum* (50%) (*I/P*) and *I. galbana* (clon T-ISO) (33%) – *P. tricornutum* (33%) – *T. suecica* (33%) (*I/P/T*). All experiments had a total concentration of 100.000 cell ml^{-1} day^{-1} and were performed in triplicate; growth and survival were estimated every 6 days.

Data analysis

Normality of data was checked with Shapiro's and Wilks' tests. The effects of density and diet on the growth of postlarvae were analyzed using the non-parametric Kruskal-Wallis test. Significant differences between treatments means were determined by the *a posteriori* Mann-Whitney U test (Zar 1999). Survival data were arcsine transformed prior to statistical analysis. To determine the effect of culture density and diet on survival, we used ANOVA and Tukey's *a posteriori* test (Sokal & Rohlf 1980). $P < 0.05$ was used as the significance level for all tests. Data were analyzed with the statistical software STATISTICA version 6.0 (Statsoft Inc., Tulsa).

Results

Effect of stocking density on postlarval culture

Figure 1 shows the growth of *M. edulis* PL cultured at different densities. At 22 days of age (6 days after the beginning of the experiment) valve length was not different among treatments; the overall mean was 502 ± 5 µm, with a growth of 30 µm day^{-1}. In the densities of 5 and 10 PL cm^{-2} we eliminated one of the 3 replicates, which were contaminated with protozoa in the first week of culture.

Between the ages of 22 and 36 days the daily growth of the PL increased rapidly; the growth rate reached 86 µm day^{-1} for the density 20 PL cm^{-2} and 102 µm day^{-1} for 5 PL cm^{-2}. At day 36, the PL cultivated at 20 PL cm^{-2} had the smallest size (1707 ± 200 µm), which was significantly different from the sizes reached by PL cultured at 5 and 10 PL cm^{-2}, whose length reached 1931 ± 260 µm and 1856 ± 213 µm, respectively. The growth rate of the PL cultured at 5 PL cm^{-2} remained at 103 µm day^{-1} at age 44 days, while the PL cultured at 10 and 20 PL cm^{-2} decreased to 40 µm day^{-1} and 47 µm day^{-1}, respectively. As a result, the mean valve length of the PL cultured at 5 PL cm^{-2} (2760 ± 396 µm) was significantly greater than the length of those cultured at 10 and 20 PL cm^{-2} (2176 ± 313 µm and 2090 ± 312 µm), respectively (Fig. 1).

Survival at 36 days of age was lower in the PL cultured at 20 PL cm^{-2} (27.5 ± 4.4%), while those maintained at 5 or 10 PL cm^{-2} were not significantly different (Table 1). At the end of the experiment the treatment of 10 PL cm^{-2} still had the greatest survival (40%), followed by 38% for the PL cultured at 5 PL cm^{-2} and 21.3% for the density of 20 PL cm^{-2} (Table 1).

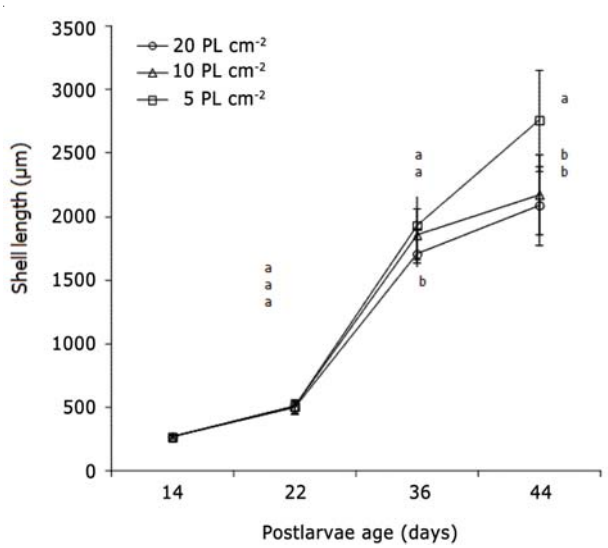

Figure 1. Growth of *Mulinia edulis* postlarvae reared in a closed system with sand at 3 culture densities (5, 10 and 20 PL cm^{-2}). Means with different letters were significantly different ($P < 0.05$) / Crecimiento de postlarvas de *Mulinia edulis* cultivadas a 3 densidades (5, 10 and 20 PL cm^{-2}) en un sistema cerrado con arena. Las medias con diferentes letras son significativamente diferentes ($P < 0,05$)

Table 1. Survival (Mean ± SD) of *Mulinia edulis* postlarvae (PL) cultured with a stocking density of 5, 10 and 20 PL cm^{-2}. Means with different letters were significantly different ($P < 0.05$) / Supervivencia (Media ± DE) de postlarvas (PL) de *Mulinia edulis* cultivadas a densidades de 5, 10 y 20 PL cm^{-2}. Las medias con diferentes letras son significativamente diferentes ($P < 0,05$)

Stocking Density/ PL Age	Survival (Mean ± SD)		
	5 PL cm^{-2}	10 PL cm^{-2}	20 PL cm^{-2}
Day 14	100	100	100
Day 36	41 ± 1 (a)	46 ± 2 (a)	28 ± 4(b)
Day 44	38 ± 10 (a)	40 ± 9 (a)	21 ± 1(a)

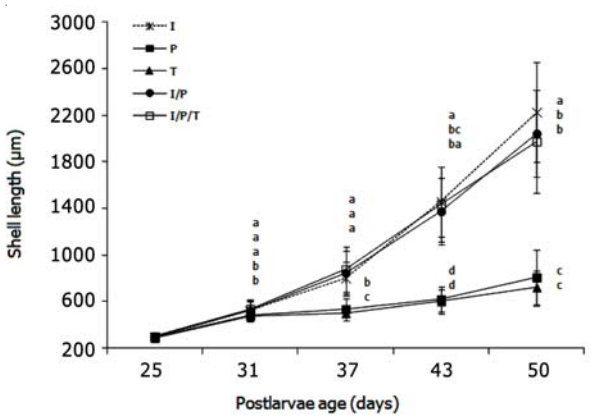

Figure 2. Growth of *Mulinia edulis* postlarvae reared in a closed system with sand with 5 diets [*Isochrysis galbana* (clone T-ISO) I, *Phaeodactylum tricornotum* P, *Tetraselmis suecica* T, *I. galbana* (clone T-ISO) – *P. tricornotum* I/P y *I. galbana* (clone T-ISO) – *P. tricornotum*– *T. suecica* I/P/T] / Crecimiento de postlarvas de *Mulinia edulis* cultivadas en un sistema cerrado con arena y alimentadas con 5 dietas [*Isochrysis galbana* (clone T-ISO) I, *Phaeodactylum tricornotum* P, *Tetraselmis suecica* T, *I. galbana* (clone T-ISO) – *P. tricornotum* I/P y *I. galbana* (clone T-ISO) – *P. tricornotum*– *T. suecica* I/P/T]

Effect of diet on postlarval culture

By day 31 (12 days of PL culture), there were already significant differences in valve length among the PL fed with different diets (Fig. 2); they remained significantly different until the end of the experiment (Table 2). The greatest mean lengths were shown by those fed with the *I/P/T* (534 ± 80 µm), *I/P* (523 ± 73 µm) and *I* (521 ± 83 µm) diets. One of the replicas of diet *T* was contaminated by protozoa in the first week of culture and was eliminated. The PL fed with the *P* and *T* diets grew less; their mean lengths were 482 ± 53 µm and 472 ± 41 µm, respectively. The mean daily growth rate for the first group of diets (*I/P/T, I/P, I*) was 39 µm, and for the second group (*P*, *T*), 31 µm.

By day 37 the difference between the 2 groups of diets was notable, and continued to increase in the following days. The daily growth with diets *P* and *T* was only 6 µm from days 31 to 37. By contrast, the diets which included *I. galbana* (*I/P/T, I/P, I*) produced much more growth in valve length; their daily growth rates were greater than 100 µm at day 50 (Fig. 2). The mean lengths at day 50 were 2225 ± 430 µm for *I*, 2042 ± 374 for *I/P* and 1974 ± 439 µm for *I/P/T*. The mean valve lengths of the PL fed with *P* and *T* were 806 ± 241 µm and 720 ± 148 µm, respectively.

The survival of the PL did not vary among diets at any point of the experiment. Although at day 50 the survival was 30% with diet *I/P/T* and 19% with *P*, the difference was not significant (Table 3).

Discussion

Larval stocking density is an important factor in determining the efficiency of hatchery production (Hurley & Walker 1996, Doroudi & Southgate 2000). This may be extended to PL culture. In this study, the greatest growth rate of PL was obtained at a density of 5 PL cm^{-2}. There are a number of similar studies which have estimated optimum culture density for the clams *Spisula solidissima similis*, *Meretrix meretrix* and *Paphia malabarica*, but the majority have dealt with the larval stage (Hurley & Walker 1996, Liu *et al*. 2006, Gireesh & Gopinathan 2008 a,b). Yan *et al.* (2006) found that larval growth decreased significantly with increasing stocking density for the manila clam, *Ruditapes philippinarum*; a density of 5-10 larvae ml^{-1}, appeared to be optimal for normal growth of manila clam larvae, while for *Mulinia edulis* the optimum density was 10 larvae ml^{-1}.

The stocking density in filtering mollusks has been generally calculated based on volumetric densities rather than area in the postlarval stage (Taylor *et al*. 1998, Doroudi & Southgate 2000). The studies of density have focused on seeds, longer than 3 mm (Hadley & Manzi 1984, Doménech 1994, Liu *et al.* 2002, Royo *et al.* 2005 a, b).

Gireesh & Gopinathan (2008a) suggested that in larvae of the clam *Paphia malabarica* an increase in density generates a greater quantity of metabolic waste which is detrimental to larval growth. Also, greater larval density produces an increase in competition for food and space

Table 2. Paired comparisons of mean length in diet treatments [*Isochrysis galbana* (clone T-ISO) *I*, *Phaeodactylum tricornutum* P, *Tetraselmis suecica* T, *I. galbana* (clone T-ISO) – *P. tricornotum* I/P and *I. galbana* (clone T-ISO) – *P. tricornotum*– *T. suecica* I/P/T] at different times using the Kruskal-Wallis test [different letters were significantly different (*P* < 0.05)] / Comparaciones pareadas de la longitud media de los diferentes tratamientos de dieta [*Isochrysis galbana* (clon T-ISO) I, *Phaeodactylum tricornotum* P, *Tetraselmis suecica* T, *I. galbana* (clone T-ISO) – *P. tricornotum* I/P e *I. galbana* (clone T-ISO) – *P. tricornotum*– *T. suecica* I/P/T] en el tiempo usando la prueba de Kruskal-Wallis [distintas letras son significativamente diferentes (*P* < 0,05)]

Diet/Time	Day 31	Day 37	Day 43	Day 50
I	a	a	a	a
P	b	b	c	c
T	b	c	c	c
I/P	a	a	b	b
I/P/T	a	a	a b	b

Table 3. Survival (Mean ± SD) of *Mulinia edulis* postlarvae (PL) fed with 5 different diets [*Isochrysis galbana* (clone T-ISO) I, *Phaeodactylum tricornotum* P, *Tetraselmis suecica* T, *I. galbana* (clone T-ISO) – *P. tricornotum* I/P and *I. galbana* (clone T-ISO) – *P. tricornotum*– *T. suecica* I/P/T] / Supervivencia (Media ± DE) de postlarvas (PL) de *Mulinia edulis* alimentadas con 5 diferentes dietas [*Isochrysis galbana* (clone T-ISO) *I*, *Phaeodactylum tricornotum P*, *Tetraselmis suecica T, I. galbana* (clone T-ISO) – *P. tricornotum* I/P e *I. galbana* (clone T-ISO) – *P. tricornotum*– *T. suecica* I/P/T]

Stocking Density/ PL Age	Survival (Mean ± SD)				
	I	P	T	I/P	I/P/T
Day 19	100	100	100	100	100
Day 37	25.2 ± 4.7	18.9 ± 6.6	22.3 ± 13.4	28.5 ± 5.3	29.6 ± 6.1
Day 43	24.6 ± 5.5	20.9 ± 4.8	26.4 ± 11.4	29.4 ± 5.0	30.2 ± 6.6
Day 50	24.7 ± 5.0	19.2 ± 4.7	24.8 ± 10.8	28.5 ± 4.4	30.0 ± 7.7

when they are fed with fixed rations of microalgae (Hurley & Walker 1996, Liu *et al* 2006, Marshall *et al.* 2010). Interspecific differences make the comparison of different species of clams difficult; for example *Cyclina sinensis* (Liu *et al*. 2002) has a larval period and size at metamorphosis very inferior to those of *M. edulis*, which allows the former species to be cultured at a postlarval density of 723 seeds cm^{-2}.

At the greatest density used in our study, 20 PL cm^{-2}, we obtained the smallest sizes and least survival; this density is not recommended in a closed system such as we used. An efficient system of re-circulation or open flow might allow a greater density or allow maximization of hatchery efficiency; this is still to be investigated.

The criteria generally used to evaluate diets are acceptability, digestibility, growth and biochemical composition (Albentosa *et al.* 1996b, Marshall *et al*. 2010). We used only the growth criterion; however the other criteria help to understand the results obtained. Our results indicate that the optimum diet, which produced the greatest shell length for seeds of *M. edulis*, is the monospecific diet of *Isochrysis galbana* (clone T-ISO), followed by the mixed diets of *I. galbana/P tricornutum* and *I. galbana/P tricornutum*/*T. suecica*. The seeds which had the least growth were those fed on monospecific diets of *P. tricornutum* and *T. suecica*.

These results are in agreement with those of Yan *et al.* (2006), who reported that larvae of the Manila clam (*Ruditapes philippinarum*) showed the greatest growth rate with a monospecific diet of *Isochrysis* spp. However, *Tetraselmis suecica* produced a low growth rate for larvae of this species (Laing *et al*. 1990, Marshall *et al.* 2010).

Nevertheless, these results are in contrast to other diet studies such as that of Albentosa *et al.* (1996a). These authors determined that for seeds of the fine clam *Ruditapes decussatus* (700 µm) the greatest growth rate was obtained with a diet of the microalga *Tetraselmis suecica*, followed by *Isochrysis galbana* (clone T-ISO), with the poorest result for *Phaeodactylum tricornutum.* Albentosa *et al.* (1993) compared diets *I. galbana* (clone T-ISO), *T. suecica* and a mixture of these 2 for the clam *Venerupis pullastra*, they found that the 50-50% mixture of the 2 algae produced the greatest growth, followed by *I. galbana* (clone T-ISO) alone.

Also, a study performed in juveniles (4mm) of the oyster *Crassostrea corteziensis*, Rivero-Rodríguez *et al.* (2007) evaluated the effect of diet on growth; they reported smaller sizes with *I. galbana* (clone T-ISO), *T. suecica* and *P tricornutum* compared to diets of *Chaetoceros calcitrans* y *Chaetoceros muelleri*. These results demonstrate the different nutritional requirements of bivalve species; thus it is necessary to determine the requirements of each species and not extrapolate to other species (Albentosa *et al.* 1996b, Rivero-Rodríguez *et al.* 2007).

The small size obtained in seeds of *M. edulis* fed with *T. suecica* may be due to the difficulty of digestion of the complex of polysaccharides and proteins of the theca (Epifanio 1979), and thus limit its nutritional value. Another possible explanation is the absence of docosahexaenoic acid (DHA; 22:6n-3) in *T. suecica* (Rivero-Rodríguez *et al.* 2007, Albentosa *et al.* 1994). In bivalve seeds, 20:5n-3 and 22:6n-3 polyunsaturated fatty acids are essential to support growth (Langdon & Waldock 1981).

According to Epifanio (1983), the most consistent factor to explain the low nutritional quality of a microalga is its indigestibility. *P. tricornutum* is a microalga which is difficult to digest; Lora-Vilchis & Maeda-Martínez (1997) found ingestion but not digestion of this species in larvae of the oyster *Argopecten ventricosus-circularis*. This fact could explain the differences in the growth of *M. edulis* with this diet.

I. galbana (clone T-ISO) diet had the best performance for PL of *M. edulis*; its high nutritional value may be related to higher ingestion, digestion, and absorption. This microalga is also an optimum diet for the clam *Paphia malabarica* (Girresh & Gopinathan 2008b) and for *Meretrix meretrix* (Tang *et al.* 2006), both studies obtained the best growth (increase in shell length) and survival with *I. galbana*. Tang *et al.* (2006) also reported that the larvae of *Meretrix meretrix* showed a preference for *I. galbana* when it was offered together with another species of microalga; this feeding behavior may also be related to essential nutrients and the morphology and size of the algae.

A diet of *I. galbana* (clone T-ISO), alone or mixed with *Chlorella* spp., produced the greatest growth and highest percentage of metamorphosized larvae in the clam *Ruditapes philippinarum* (Yan *et al.* 2006). The same result was found for *Paphia malabarica* fed with a mixture of the microalgae *I. galbana* and *Nannochloropsis salina* (Gireesh & Gopinathan 2008b). Bivalve larvae are generally fed with a mixture of microalgae species to provide a better nutrient balance (Southgate 2003).

Helm & Laing (1987) obtained good growth with *I. galbana* (clone T-ISO) for larvae of the clams *Tapes semidecussata* and *Mercenaria mercenaria*, but not for the oyster *Crassostrea gigas* or the mangrove oyster, *Crassostrea rhizophore*. *I. galbana* (clone T-ISO) can be beneficial as a constituent species of a mixed algal diet, as shown by significantly improved growth of the larvae of three of the four bivalves tested.

A number of studies have demonstrated that both the quantity and quality of food affect the growth and survival in the larval stages (Hurley *et al.* 1997), fixation and growth of clam seeds (Castagna & Kraeuter 1981). These results also illustrate the importance of feeding the correct ratio of different species in any food mixture to give the most efficient utilization of the diet and to obtain the best growth.

We did not detect significant differences in survival with different diets after 50 days of culture. However, our results showed that mixed diets gave better mean survival than monospecific diets. This was not true for *Meretrix meretrix*; those fed with *I. galbana*, had greater survival than those with mixed diets (Liu *et al.* 2006).

The presence of *Isochrysis galbana* in the best diet in our study may be explained by its high concentration of proteins and lipids (Albentosa *et al.* 1994), while *Tetraselmis* has a greater quantity of carbohydrates, which along with *Tetraselmis* may have provided other compounds which improved survival.

Mulinia edulis is a species resistant to management, since its survival was not affected by the diet, or stocking density. The high percentages of survival (up to 25%) obtained in the experiment on food concentration confirmed this.

The differences obtained in the growth of seeds with different diets and culture density suggest that to optimize PL culture, metamorphosis and later culture of *Mulinia edulis* seeds, they should be fed a monospecific diet of the microalga *Isochrysis galbana* (clone T-ISO), and cultured at a density of 5 PL cm^{-2}. As we hypothesized, a low stocking density resulted in a better growth rate for the PL, but the *Isochrysis galbana* unialgal diet had a better performance than the mixed diets that we had expected.

Although these factors were studied independently, the results for each of them represent an important advance in the development of a protocol of optimum culture for seeds of *M. edulis* with good growth and a high survival percentage; and thus represent a key to the future commercial culture of the species.

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