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*Research Article*

# Grown-out of seeds of the taquilla clam *Mulinia edulis* (King & Broderip, 1832) in the subtidal zone in northern Chile and in the intertidal zone in southern Chile

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**ABSTRACT.** Seeds of the clam *Mulinia edulis* produced in a hatchery were grown in bottom cages in the subtidal zone of Tongoy Bay (northern Chile) and in the intertidal zone of Chullec (Chiloé Island, southern Chile), to compare the growth and survival of small and large seeds of the same production in different environments and latitudes. Seeds were shipped twice to Chullec, once in summer and once in winter. Previous experiments with seed transportation (14.2 ± 2.4 mm) showed that they are able to survive more than 24 h out of water. The growth rate of both large and small seeds was greater in Tongoy than in Chullec, reaching maxima of 1.24 mm month$^{-1}$ in the small seeds of Tongoy and 1.06 mm month$^{-1}$ in the same group in Chullec. The growth rates obtained for *M. edulis* were similar to those described for other cultured clams. Seeds grown in Chullec presented a significantly greater weight by size than the seeds grown in Tongoy. Survival after 12 months was 38% and 88% in Tongoy and 19.4% and 37.3% in Chullec for small and large seeds, respectively. Seeds produced in hatcheries are able to withstand long travel (1,360 km) and grow in the intertidal zone of southern Chile, where the operation of the culture is simple and cheap. The growth curve presents an inflection point around 20 mm length, probably related to the age at sexual maturity. Growing out seeds in the intertidal zone in southern Chile is seen as a new activity for artisanal fishermen.

**Keywords:** clam seeds, subtidal, intertidal culture, growth, survival, Chile.

## Engorda de semillas de la almeja taquilla *Mulinia edulis* (King & Broderip, 1832) en la zona submareal en el norte y en la zona intermareal en el sur de Chile

**RESUMEN.** Semillas de la almeja *Mulinia edulis* producidas en el Hatchery de Pesquera San José (Tongoy) fueron engordadas en sistemas de fondo en la zona submareal de bahía Tongoy e intermareal en Chullec (Chiloé), para comparar el crecimiento y la supervivencia de semillas pequeñas y grandes de una misma producción en diferentes ambientes y latitudes. Se realizaron dos envíos de semillas a Chullec, uno en verano y otro en invierno. Experimentos de simulación del transporte de semillas (14,2 ± 2,4 mm) muestran que éstas sobreviven al transporte por más de 24 h. La tasa crecimiento de las semillas grandes y pequeñas fue mayor en Tongoy que en Chullec, alcanzando un máximo de 1,24 mm mes$^{-1}$ en las semillas chicas de Tongoy y de 1,06 mm mes$^{-1}$ para el mismo grupo en Chullec. Las semillas engordadas en Chullec presentaron un peso significativamente mayor e igual talla que las de Tongoy. La supervivencia, después de 12 meses, de las semillas pequeñas cultivadas en Tongoy fue de 38% y de 19,4% en Chullec y de las semillas grandes de 88% y 37,3% respectivamente. Las semillas producidas en hatchery son capaces de sobrevivir a largos traslados (1.360 km) y de crecer en la zona intermareal del sur de Chile, donde el manejo de los cultivos es más simple y barato. La tasa de crecimiento presentó un punto de inflexión a los 20 mm, que se relaciona con la edad de madurez sexual. La engorda de semillas en la zona intermareal del sur de Chile se vislumbra como una nueva actividad económica para los pescadores artesanales.

**Palabras clave:** semillas de almejas, cultivos intermareales, cultivos submareales, crecimiento sobrevivencia, Chile.

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## INTRODUCTION

*Mulinia edulis* (King & Broderip, 1832) is known as taca, taquilla, culeca or sweet clam. It is found in the Pacific from Callao, Peru to the Strait of Magellan in southern Chile (Oliva *et al.*, 2005; Jaramillo *et a.l*, 2008) and in some areas of the Argentinean Patagonia in the Atlantic (López & Cruz, 2007). This clam lives buried in sand and sand-mud bottoms (Jaramillo *et al.*, 2007); it is found in protected coasts both in subtidal zones and in the lower intertidal zone, between 2 and 17 m depth in Coquimbo region (Stotz *et al.*, 2008). This species is tolerant to high population densities and even to low oxygen content (Oliva *et al.*, 2005; Stotz *et al.*, 2008). According to Stotz *et al.* (2008), the minimum legal size (5.5 cm) is reached at age 13 months in the zone of Coquimbo, while in Región de Los Lagos (Jaramillo *et al.*, 2008) this size is reached at age 8 to 12 years.

*M. edulis* is one of the six clam species currently exploited by artisanal fishermen. It represents about 17% of the clam landings during the past five years, and is the third species in importance in national landings (SERNAPESCA, 2010).

Clam extraction is regulated in Chile by closing the access to certain fisheries and setting a minimum legal extraction size of 5.5 cm for all clam species (Decree Nº683, 1980, Ministry of Economy). It is relevant to mention that there are currently no commercial clam cultures in Chile; all clams come from artisan landings, part of which is sold fresh and the rest is derived to the processing industry, mainly for exportation.

Techniques for the production and growth of seeds in natural environments have been described in Europe, particularly in Spain and Italy, and in the United States, for several species, of both native and introduced clams: *Ruditapes philippinarum*, *R. decussatus*, *Venerupis pullastra*, and *Mercenaria mercenaria* (Mattei *et al.*, 1990; Pellizato, 1990; Cigarría & Fernández, 2000; Abella, *et al.*, 2001; Kraeuter & Castagna, 2001; Royo *et al.*, 2005a, 2005b).

Currently, small clams, commercially known as baby clams (3 cm shell length), are a highly sought-after product in the European market, where they are consumed fresh, frozen or canned. The fishery regulations in Chile forbid the extraction of small clams from natural banks. This regulation is not applicable for clams produced in hatcheries and the harvest size can be that of baby clams.

The intertidal zone of southern Chile presents large variations during the spring tide, creating broad extensions of sandy beach, appropriate for clam grow-out. One of the main characteristics of the use of this zone is the fact that the culture techniques are of easy implementation, and have lower costs compared to suspended and subtidal bottom systems, which require boats and divers. The aim of this paper is to compare the growth and survival of *M. edulis* clam seeds in the subtidal zone in Tongoy Bay (northern Chile) and in the intertidal zone in Chullec Bay (southern Chile) and to determine the time required for the production of small or baby clams.

## MATERIALS AND METHODS

### Seed production

*Mulinia edulis* has separate sexes, the mature gonad has a dark purple colour in the females, and in young clams it is possible to observe it through the shell. *M. edulis* clam larvae were cultured in mass systems in the hatchery of Pesquera San José S.A., located in Tongoy Bay, Coquimbo (30°15’27”S, 71°29’33”W) (Fig. 1). The larvae were settled in a fine-grained sand substrate at a temperature of 17° ± 1º C for 50 days until they reached a size of 2 mm; then kept in the nursery until they reached 12 mm (Oliva *et al.*, in press). The seeds were settled in summer in the subtidal zone of Tongoy and in summer and winter in the intertidal zone of Chullec (Quinchao Island, Chiloé, 42°28'20"S, 73°32'10" W). The first shipment to Chullec was made by land (app. 37 h) and the second was made by air (app. 11 h). Natural banks of the species are found in Tongoy and in Chiloe Island.

### Effect of shipment time

The effect of shipment time on seed survival under standard conditions of ground shipping was evaluated. Three replicates of 10 seeds with an average size of 14.2 ± 2.4 mm were distributed for each shipment time treatment (Control, 1, 2, 4, 8, 24, 48 and 72 h). The seeds were placed between wet sponges in an isothermal box with cold packs, to maintain the temperature during the experiment (13.75 ± 0.48°C). The seeds on each shipment treatment were put on fine sand and the burying success was evaluated at 2, 10, 30 and 120 min.

### Seed grow-out experiments

The Tongoy Bay experimental site was chosen in a sector of fine sand and mud which belongs to an aquiculture concession of the, Pesquera San José, with a mean depth of 14 m. In the Chullec site the experiments were mounted in a shallow bay in a sector with fine sand and clay which had a maximum depth

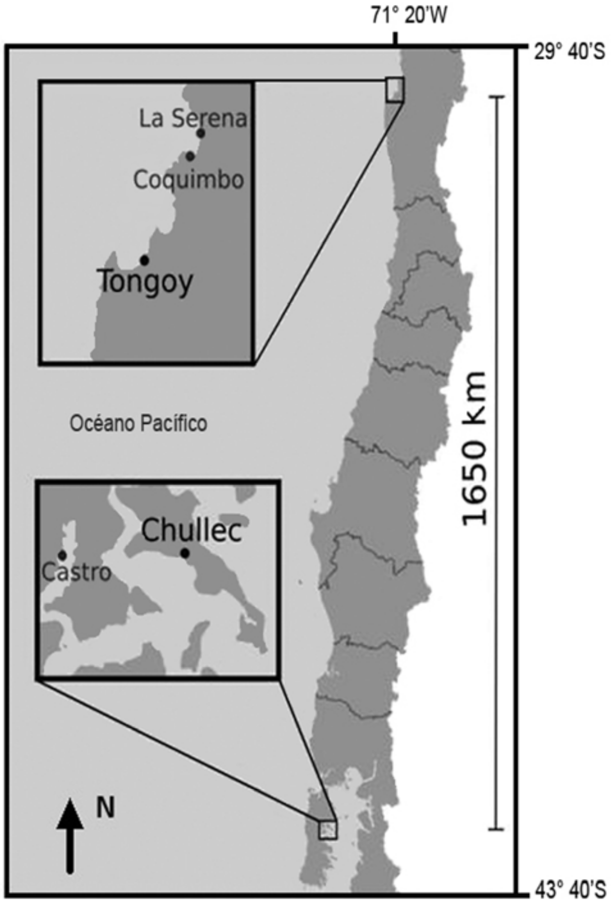

**Figure 1.** Geographical location of experimental areas for the evaluation of growth of the clam seed of *Mulinia edulis* between February 2004 and February 2005.

**Figura 1.** Ubicación geográfica de las áreas experimentales para la evaluación de crecimiento de semillas de la almeja *Mulinia edulis* entre febrero 2004 y febrero 2005.

of 7 m. Only during the spring tides did the culture systems remain out of water for a few hours per day.

We sent two shipments to Chullec with seeds produced in the hatchery in Tongoy. The hatchery is located 1,360 km from Chullec. The first group of 12,200 seeds belonging to the same batch was shipped by ground during summer (February 18, 2004). Seeds were separated by size; large seeds (3,500 seeds with an average size of 22.2 ± 2.4 mm) were cultured in a cage of 2 $m^2$ at an initial density of 1,750 seed $m^{-2}$; small seeds (8,900 seeds of 17.1 ± 2.8 mm) were split into three cages with an initial density of 1,450 seed $m^{-2}$. The same experiment was mounted simultaneously in Tongoy Bay at a depth of 14 m. The cages were fixed to the bottom and marked for later evaluations. A second shipment to Chullec of seeds of 7.5 ± 1.2 mm was sent by air during winter (June 22, 2004) to compare the growth with summer conditions. The seeds were placed in two cages with an initial density of 1,450 seeds $m^{-2}$. The cages of both shipments were placed in the lower intertidal zone.

The cages were designed with a ½” plastic-covered metal wire. An external layer of rigid plastic mesh with pore opening of 1 cm was put in the base. A second waterproof layer of plastic sackcloth was placed in the base and sides to retain the sand and to avoid the access of predators. Samplings were performed during low tides in Chullec and with the help of a boat and divers in Tongoy Bay. In each revision, length, height and thickness of the *M. edulis* seeds were measured with a vernier calliper. Weight was measured with a Sartorius B-160 weighing scale in Tongoy Bay and an O’Haus Portable Plus Model C505S scale in Chullec. Both scales were calibrated and have a precision of 0.1 mg. Samples of 100 seeds were taken from each cage monthly the first three times and once every two months thereafter. After measuring, the seeds were put back into the cages. Survival was evaluated at month four (June), eight (October) and twelve (February), by counting the shells of dead clams in the cages.

### Data analysis

To compare valve length between the groups of Tongoy Bay and Chullec a Student’s t-test was calculated separately for each monthly comparison, after an analysis of variance homogeneity was tested using the F-test of variance for two samples (Zar, 1996).

For the analysis of the size/weight relationship 30 random individuals were chosen each time from each cage. The homoscedasticity was tested with Bartlett’s test. Weights were transformed to natural logarithms for further analysis. Finally, an analysis of covariance was performed to test the difference between the slopes of the growth rates among localities (Zar, 1996).

## RESULTS

### Effect of shipment time

The seeds of *M. edulis* of 14 ± 2 mm were capable of being out of water (under shipment conditions) for 8 h and 100% buried themselves within 30 min of being put on a substrate of sand covered by 2 cm of water (Table 1). After 24 h of exposition to shipment conditions, the ability to bury themselves during the first two hours decreased to 90%. After 72 h only 60% of the seeds were able to bury in the sand (Table 1).

**Table 1.** Shipment time in a humid environment and burying success (%) of *Mulinia edulis* seeds evaluated at 2, 10, 30 and 120 min after seeding on sand, time required to bury and survival of *Mulinia edulis* seeds.

**Tabla 1.** Tiempo de traslado en ambiente húmedo y éxito de enterramiento (%) de semillas, de *Mulinia edulis*, evaluado a los 2, 10, 30 y 120 min después de la siembra en arena.

| | Burying success (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Shipment time (h) | 2 min (mean ± SD) | | 10 min (mean ± SD) | | 30 min (mean ± SD) | | 120 min (mean ± SD) | | Temperature (°C) |
| Control | 60 | 10 | 90 | 10 | 100 | 0 | 100 | 0 | 14 |
| 1 | 70 | 10 | 86.6 | 5.8 | 100 | 0 | 100 | 0 | 13.9 |
| 2 | 76.6 | 5.8 | 96.6 | 5.8 | 100 | 0 | 100 | 0 | 13.5 |
| 4 | 70 | 10 | 80 | 10 | 100 | 0 | 100 | 0 | 13.8 |
| 8 | 76.6 | 5.8 | 86.6 | 5.8 | 100 | 0 | 100 | 0 | 14.3 |
| 24 | 73.3 | 15.3 | 86.6 | 15.3 | 90 | 10 | 90 | 10 | 12.8 |
| 48 | 50 | 10 | 63.3 | 5.8 | 70 | 10 | 86.6 | 7.1 | 13.5 |
| 72 | 36.6 | 11.5 | 46.6 | 5.8 | 53.3 | 5.8 | 60 | 11.5 | 14.2 |

The seeds that were unable to bury themselves within 2 h presented very little activity, sitting on top of the sand with their siphons out.

### Seed grow-out experiments

The small seeds cultured in summer presented sustained growth in both localities, however the seeds kept in the intertidal of Chullec had a lower growth rate ($P < 0.05$), reaching an average shell length of 29.8 mm, which means an increase of 12.7 mm year$^{-1}$; while in Tongoy the clam seeds in the subtidal reached a size of 31.9 mm, which is an increase of 14.9 mm year$^{-1}$.

The seeds grown in Chullec had a higher growth rate than their counterparts in Tongoy during the first eight months, after which the growth rate of Tongoy seeds accelerated, reaching a greater average final size (Fig. 2a), with a significant difference between the groups of Chullec and Tongoy ($P = 3.04\text{x}10^{-15}$). From November onwards the cages of Chullec were covered with the green alga *Ulva lactuca*, which produced a lower exchange of water in the cages.

Growth in weight was five-fold in the subtidal of Tongoy and four-fold in the intertidal of Chullec. As with shell length growth, weight growth was significantly greater in the seeds of Tongoy during the last months of the experiment ($P < 0.001$, Fig. 2b).

The growth of larger clams (22.2 mm shell length) was similar in both locations during the first six months (Fig. 3a). From the eighth month on the seeds fattened in Chullec showed less growth, with a significant difference ($P < 0.01$) compared to the seeds that grew in Tongoy. By the end of the experiment the seeds of Chullec reached a final average size of 28.3 mm, with an increase of 6.1 mm. The seeds that grew in the subtidal zone of Tongoy had a slightly larger valve length of 31.2 mm, which represents a growth of 9 mm for the evaluation period.

Table 2 shows the growth rate of *M. edulis* seeds and four other clam species kept in sandy substrates. The growth rate observed ranged from 0.53 to 3.22 mm per month. In general, smaller seeds had the greatest growth rate. We observed the presence of a purplish coloration in the mid-region of the shell in individuals larger than 26 mm (some even smaller), and during the growth evaluation some seeds evacuated their gametes (males and females).

The growth in weight of the seeds that were initially larger (1.64 ± 0.12 g) followed the same pattern both in Tongoy and Chullec until the sixth month (August). In the following months the weight of the seeds in Tongoy was significantly greater (Fig. 3b); the mean weight increment of the seeds of Tongoy was 3.5 g, while in Chullec it was 2.75 g. In the final evaluation of the experiment (February 2005), small seeds reached the same final weight as large seeds, both in Chullec and Tongoy (Figs. 2b and 3b).

About 70% of the large seeds and 40% of the small seeds (2 cm) of Tongoy were found to be sexually mature. We even found ovocytes and sperm in the buckets used for weight and size sampling. We also detected the presence of mature specimens in Chullec.

The length/weight relationship in both sites presented an exponential fit (Fig. 4). An analysis of covariance showed significant differences ($P < 5.06\text{x}$

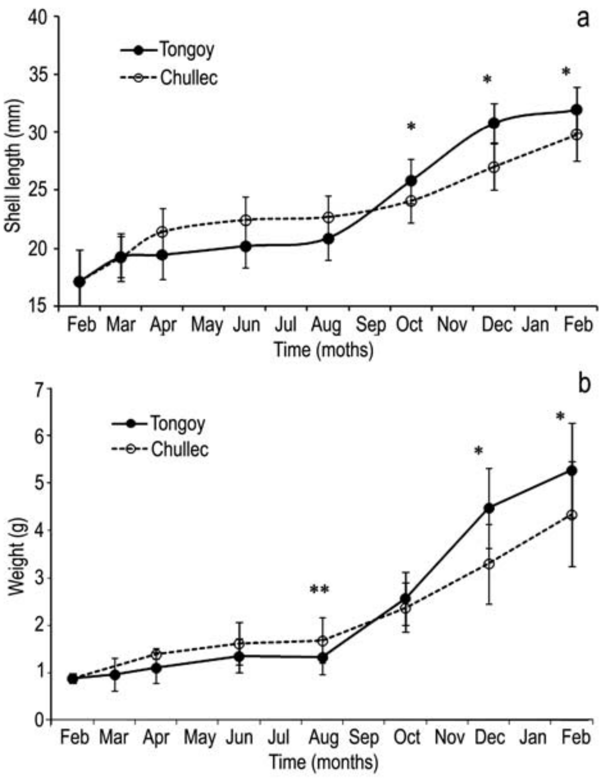

**Figure 2.** Growth in length (a) and weight (b) of seed clams with initial size of 17.1 mm grown out in the subtidal of Tongoy Bay (black circles) and the intertidal of Chullec (white circles) between February 2004 and February 2005. Vertical lines indicate standard deviation. An asterisk (*) represents a significant difference of $P < 0.01$ and two asterisks (**) represents significant difference of $P < 0.05$.

**Figura 2.** Crecimiento en longitud (a) y peso (b) de semillas de almejas con tamaño inicial de 17,1 mm, engordadas en el submareal de Bahía Tongoy (círculos negros) y en el intermatral de Chullec (círculos blancos) entre febrero de 2004 y febrero de 2005. Las líneas verticales indican desviación estándar. Un asterisco (*) representa diferencia significativa de $P < 0,01$ y dos asteriscos (**) representa diferencia significativa de $P < 0,05$.

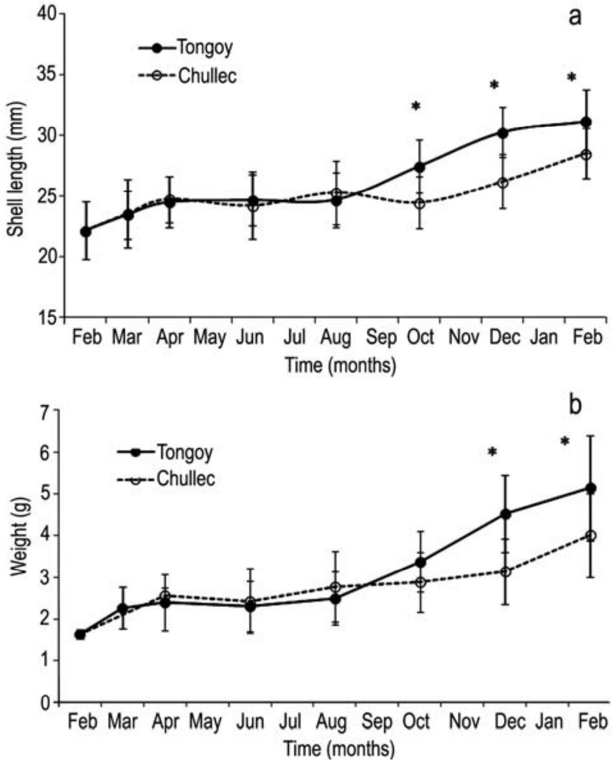

**Figure 3.** Growth in length (a) and weight (b) of seed clams with initial size of 22.2 mm grown out in the subtidal of Tongoy Bay (black circles) and in the intertidal of Chullec (white circles) between February 2004 and February 2005. Vertical lines indicate standard deviation. An asterisk (*) represents significant difference of $P < 0.01$.

**Figura 3.** Crecimiento en longitud (a) y peso (b) de semillas de almejas con tamaño inicial de 22,2 mm, engordadas en el submareal de bahía Tongoy (círculos negros) y el intermareal de Chullec (círculos blancos) entre febrero 2004 y febrero 2005. Las líneas verticales indican desviación estándar. Un asterisco (*) representa diferencia significativa de $P < 0,01$.

$10^{-8}$) between the slopes of the transformed data, which indicates that the seeds of Chullec were able to incorporate more biomass than the seeds of Tongoy for the same shell length.

In the first survival survey (fourth month) we detected a drastic mortality in the small seeds sent from Tongoy to Chullec, which registered a survival of 28.1%, compared to the seed cultured in Tongoy over the same period, which had 50.1% survival (Fig. 5a). Some of the smaller seeds suffered damage in their shells and some stench was present when the experiments where mounted. An *in situ* evaluation revealed that several clams had not buried after 12 h, which is a sign that they were affected by their transportation.

Over the course of the next eight months survival continued to decrease in both sites (Fig. 5a); however there was markedly lower survival in the seeds of Chullec, which reached only 19.4%, compared to the seeds of Tongoy, which had 37.6% survival at the end of the experiment. On the last sampling date it was found that the two first cages had lost sand (50%) and a nearby cage was covered with sand (embankment), both of which increased mortality.

The *in situ* evaluation of the first shipment showed that large seeds buried quickly (before 12 h) and their siphons could be clearly seen in the substrate. Unlike

**Table 2.** Comparison of the growth rate of clam seed with different input sizes in bottom culture systems.

**Tabla 2.** Comparación de la tasa de crecimiento de semillas de diferentes especies de almejas en sistemas de cultivo de fondo.

| Clam species | Initial size (mm) | Final size (mm) | Growth rate (mm month$^{-1}$) | Time (months) | References |
|---|---|---|---|---|---|
| *Mulinia edulis* (Chullec winter shipping) | 7.47 | 29.34 | 2.73 | 8 | This study |
| *Mulinia edulis* (Chullec-large) | 22.2 | 28.55 | 0.53 | 12 | This study |
| *Mulinia edulis* (Chullec-small) | 17.09 | 29.81 | 1.06 | 12 | This study |
| *Mulinia edulis* (Tongoy-large) | 22.2 | 31.2 | 0.75 | 12 | This study |
| *Mulinia edulis* (Tongoy-small) | 17.09 | 31.96 | 1.24 | 12 | This study |
| *Ruditapes decussatus* | 1.4 | 4.1 | 0.67 | 4 | Pérez-Camacho (1980) |
| *Ruditapes decussatus* | 11.7 | 30.4 | 2.34 | 8 | Pérez-Camacho & Cuña (1987) |
| *Ruditapes philippinarum* | 4.73 | 35 | 1.78 | 17 | Di Muro *et al.* (1990) |
| *Ruditapes philippinarum* | 7.4 | 28.8 | 1.78 | 12 | Pérez-Camacho & Cuña (1985) |
| *Ruditapes philippinarum* | 9.87 | 25.1 | 2.17 | 7 | Royo *et al.* (2005a) |
| *Ruditapes philippinarum* | 9.87 | 23.32 | 1.92 | 7 | Royo *et al.* (2005a) |
| *Ruditapes philippinarum* | 11.24 | 30.1 | 3.14 | 6 | Royo *et al.* (2005b) |
| *Ruditapes philippinarum* | 11.3 | 37.39 | 2.07 | 13 | Royo & Ruiz-Azcona (2005) |
| *Ruditapes philippinarum* | 13.7 | 39.5 | 3.22 | 8 | Pérez-Camacho & Cuña (1987) |
| *Ruditapes philippinarum* | 30.1 | 35.2 | 0.85 | 6 | Royo *et al.* (2005b) |
| *Venerupis pullastra* | 3.2 | 29 | 2.85 | 12 | Pérez-Camacho (1980) |
| *Venus antiqua* | 6 to 10 | 35 | 1.93 to 1.66 | 15 | Bustos (2003) |

the small seeds group, large seeds had more than 85% survival during the first eight months in both sites (Fig. 5b). In the last sampling, however, the clams of Chullec had a sharp decrease, reaching 37.3% survival by the end of the experiment. This mortality may have been produced after the second sampling because the cages lost sand. Clams kept in the subtidal zone of Tongoy had 88% survival at the end of the experiment.

The first evaluation of survival showed a high mortality in both seed groups (fourth month). In Table 3 it can be seen that dead seeds in the experiments were not larger than the seeds measured at the beginning of the experiment (t test, $P < 0.05$). The presence of predators was detected in the cages of both Chullec and Tongoy. In the cages of Chullec it was found only a decapod predator, *Cancer setosus* and the scavenger gastropod *Nassarius gayi*. During the first sampling 6.6% of the large seeds had been perforated. There was a greater variety of predators in Tongoy; two gastropod species, *Argobuccinum (Priene) scabrum* (hairy snail) and *Oliva peruviana*; one asteroidean *Meyenaster gelatinosus*; and two species of decapod crustaceans, *C. setosus* and *Pilumnoides perlatus*. Among the sampled cages, 50% of the clams counted as dead were found with a shell perforation.

In the second shipment of seeds to Chullec, (winter) the initial shell length of seeds of *M. edulis* was 7.47 ± 1.2 mm. Comparing the growth of the seeds of the second shipment (June 2004) to the seeds of the first shipment (February 2004), it can be seen that both clam populations reached the same size at the end of the experiment (Fig. 6), with no significant difference ($P < 0.136$). The seeds introduced in June 2004 (winter) had a good growth rate during the period of study, reaching a net increase of 21.9 mm over eight months, with an average growth rate of 2.73 mm month$^{-1}$.

## DISCUSSION

### Effect of shipment time

The results of this study show that *Mulinia edulis* clam seeds of 14 mm length are able to resist periods of up to 8 h out of water during shipment without mortality and with the capacity to bury themselves within 30 min after putting them in contact with sand. Periods of more than 24 h of shipment can reduce the ability of

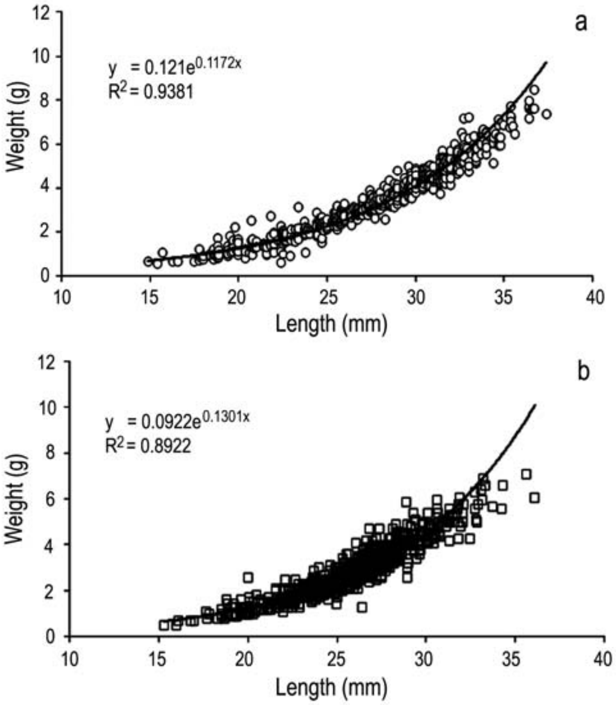

**Figure 4.** Length-weight relationship of clam seed grown out in subtidal cages in Tongoy Bay (a, open circles) compared with seeds that grew in intertidal cages in Chullec (b, open squares).

**Figura 4.** Relación longitud-peso de las semillas engordadas en jaulas en el submareal en bahía Tongoy (a, círculos abiertos) y en el intermareal de Chullec (b, cuadrados abiertos).

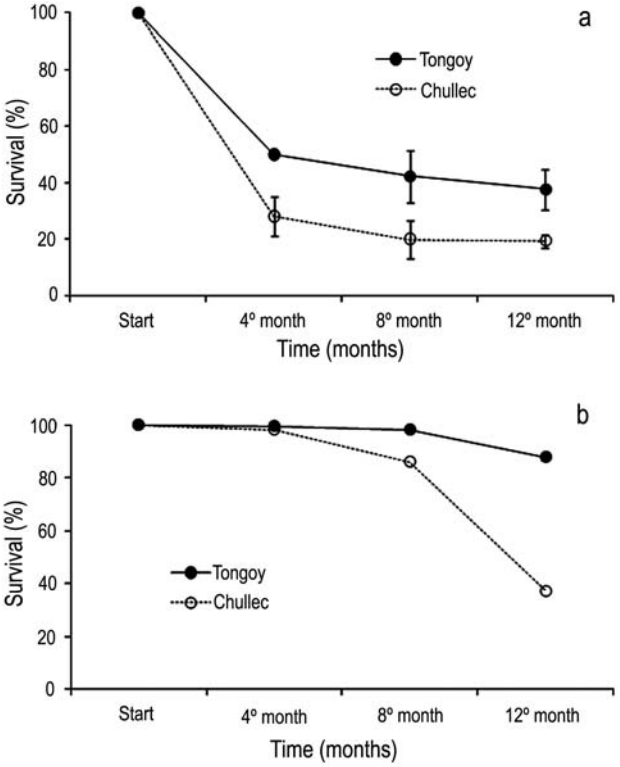

**Figure 5.** Survival of *Mulinia edulis* clam seed grown out in bottom cages in the subtidal zone of Tongoy Bay and in the intertidal zone of Chullec, transplanted in the first shipment of february 2004. a) Large seeds (17.1 mm), and b) small seeds (22.2 mm).

**Figura 5.** Supervivencia de las semillas de la almeja *Mulinia edulis* engordadas en jaulas de fondo en el submareal de bahía Tongoy y en el intermareal de Chullec, ingresadas en febrero 2004. a) Semillas grandes (17,1 mm), y b) semillas chicas (22,2 mm).

the seed to bury by 10% when they were put in a sandy substrate and evaluated after 2 h. Parada & Peredo (2005), working with the freshwater clam *Diplodon chilensis* with the aim of relocating individuals as management strategy, reported that individuals of shell length greater than 50 mm presented low resistance to shipment times longer than 4 h, with survival rates under 20%. Solis & Heslinga (1989) verified that the use of pure oxygen in the transportation systems for *Tridacna derasa* clam seeds produced better survival in the range of 16 to 48 h compared to systems exposed to only atmospheric air. Braley (1992), aiming to increase the survival of *Tridacna gigas*, used pure oxygen and added antibiotics (streptomycin sulphate, 25 ppm); he reported a survival rate of 98% after 30 h of exposition to air. For larger seeds of *T. gigas* (5.5 months old), times above 40 h gave 64% survival after four days of evaluation post re-immersion. Our data on 14 mm seeds showed survival above 85% after 48 h when seeds were subjected to experimental shipment conditions as defined above. Those seeds that did not bury themselves after 120 min are left exposed to depredation by decapods and gastropods.

Most clam species live buried in the subtidal zone and are not well adapted to being out of water for long periods, unlike other bivalves which live in the intertidal zone (Nichitta & Ellington, 1983; Velasco & Navarro, 2002) that have physiological responses to air exposition conditions (Widdows & Shick, 1985). Seeds of *M. edulis* of 14 mm were able to survive over 6 h, which is about the period of spring tides. *M. edulis* seeds show a good capacity to bury in a sandy substrate after shipment in humid conditions. This reinforces that transplantation of seeds reared in hatcheries will be successful.

From an aquaculture production point of view, the distance between a clam hatchery (or nursery) and the final grow-out sites must be kept to a minimum in order to avoid the mortality associated with transport

**Table 3.** Initial seed length and length of dead seed clams at the first control (four months) in bottom cages in Tongoy Bay (subtidal) and Chullec (intertidal).

**Tabla 3.** Longitud inicial de semillas y longitud de las conchas de semillas muertas al primer control de mortalidad (cuatro meses) en jaulas de fondo en bahía Tongoy (submareal) y Chullec (intermareal).

| Locality | Size group | Initial seed length ( mean ± SD) mm | Dead seed length ( mean ± SD) mm | n |
|---|---|---|---|---|
| Chullec | small seeds | 17.1 ± 2.8 | 16.4 ± 2.2 | 150 |
| | big seeds | 22.2 ± 2.4 | 19.7 ± 2.6 | 50 |
| Tongoy | small seeds | 17.1 ± 2.8 | 17.5 ± 1.8 | 40 |
| | big seeds | 22.2 ± 2.4 | 20.1 ± 2.7 | 50 |

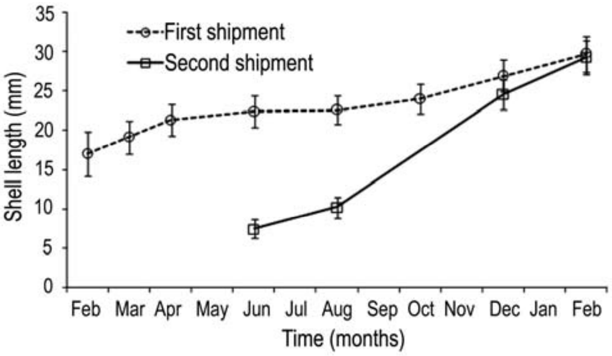

**Figure 6.** Growth of *M. edulis* seeds transplanted from Tongoy Bay to Chullec in two seasons; the first shipment in February 2004 and the second shipment in June 2004.

**Figura 6.** Crecimiento de semillas de *M. edulis* transplantadas desde bahía Tongoy a Chullec en dos estaciones del año. El primer envío fue en febrero 2004 y el segundo envío fue en junio 2004.

time. For shipment of clam seeds particular care must be taken in the selection and preparation of the rearing site; an adequate transportation method considering time and conditions must also be selected (Royo *et al*., 2005a; Gomez & Mingoa-Licuanana, 2006). The substrate composition must be taken into account (Royo *et al*., 2005a; Gomez & Mingoa-Licuanana, 2006), such as the sand grain size (middle grain, fine grain, mud-sand), and the time of the year in which the shipment will be made.

One relevant result of this study is that artificial production clams (from a hatchery) are able to resist stress conditions and growth in the intertidal zone of southern Chile.

## Growth and culture conditions

The culture of clams in an intertidal zone may have advantages for the management of seeds, associated mainly with lower operation costs, compared to subtidal cultures, as in the case of Tongoy. Subtidal culture involves costs related to the use of specialized vessels and personnel (divers). Moreover, as happened in the spring months in Tongoy, there were occasions when it was impossible to work due to bad oceanographic conditions. During summer, Tongoy has strong currents in its water column, which may have had incidence in the loss of sand in some cages and the burying of others, as happened with some of the cages with small seeds.

The cages used in this experiment were difficult to operate in the subtidal zone, mainly when they needed to be extracted from the bottom for growth evaluation, harvest or management of predators. Thus the use of cages a subtidal environment in clam rearing is not a good technological alternative, mainly due to their difficulty of manipulation and to its cost. Clam seed culture in an intertidal zone was simpler, taking into account that it depends on the tides. Unlike a subtidal system, it is easier to check several cages in a short time. This makes feasible early detection of the effect of bottom currents and embanking, overcrowding management (when seeds concentrate in one part of the cage) and even renewal of the sand. Another advantage of working in the intertidal is the ability to exclude predators, mainly crabs, as reported in other clam cultures (Di Muro *et al*., 1990; Pellizzato *et al*., 1990; Royo *et al*., 2005b)

Rearing in the intertidal zone presents more operational advantages than the subtidal zone, however in this study there were other variables not evaluated, such as the effect of the change of tides during spring tide and the effect of the increase of surface temperature in the spring – summer seasons, the decrease of temperature in winter and the effect of the rains in the case of the south of Chile. All those variables might have produced some degree of stress in the clams.

Bustos & Olavarría (2000) and later Bustos (2003) mentioned that the Chilean clam *Venus antiqua* takes about 15 months to reach a length of 35 mm, starting at sizes between 6 and 10 mm, which indicate a growth rate between 1.66 and 1.93 mm month$^{-1}$ (Table 2). Present results showed growth rates between 0.75 and 0.53 mm month$^{-1}$ for Tongoy and Chullec, respectively, when the clams were evaluated from 20 to 25 mm. This trait can be seen in other clams, as in the case of *Ruditapes philippinarun* in Spain (Royo & Ruiz-Azcona, 2005; Royo *et al.*, 2005b) and in Italy (Di Muro *et al.*, 1990). In the experiment *M edulis* seeds with an initial size of 7.47 mm showed a better performance than *Ruditapes philippinarum* seeds of the same size.

Only *Venerupis decussata* presents low growth rates at sizes below 20 mm (Pérez-Camacho, 1980). However, the seeds of taquilla clam can be reared from 5 to 30 mm over one year as it has been shown, being possible that the culture time may be reduced by optimizing the technology.

Taquilla clams presented a rapid growth up to a size of about 20 to 25 mm and a slow growth rate once they reached a size close to 30 mm. In the last revision at the end of the experiment (February, 2005), some clams began lying eggs and ejaculating during the morphometric measurements, particularly in Tongoy. The change in growth rate is associated with the sexual maturity. Several authors have determined the size of population sexual maturity in different localities in Chile (Orellana, 1980; Brown *et al*., 1999; Jaramillo *et al.*, 1998, 2008; Stotz *et al.*, 2008) described mature clams in individuals under 20 mm.

### Mortality of seeds

In the first revision of mortality, in the fourth month of the experiment, high mortality was found due to the effect of seed transport. Another cause was that small clams were weaker than larger ones. It is noteworthy that small clam seed cultured in Tongoy had decreased survival at the fourth month of evaluation, even though in Tongoy shipment time did not exceed 2 h. Thus to perform a survival analysis, care must be taken to consider the initial number of living seeds introduced to the system; in this case not all were alive, and a fraction were in bad condition. Furthermore, it must be mentioned that there are other causes inherent to the place of rearing which may account for the initial mortality, such as quality of the water, pollution and density of seeding, among others (Pellizzato *et al.,* 1990; Royo *et al*., 2005a, 2005b).

The use of protection meshes reduced considerably the loss of biomass in the grow-out phase; the dynamics of the sediment of the work area must be known, because part of the mortality in the Tongoy site was due to the embanking of the culture cages, with the consequent death of the seeds that could not extend their siphon.

The selection of the clam grow-out site is important, since granulometry (sand, sand-mud, mud-sand), degree of sinking, accessibility to the culture and slope of the beach are elements to consider for a good result. Peña *et al*. (2005), found that only 4% of the intertidal zone of Río Piedras (Huelva-España) was appropriate for the culture of *Ruditapes decussates*; the rest of the zone required preparation. Recently, in Chile the use of intertidal zones of the Región de Los Lagos is being evaluated, however not all places present the correct granulometric, bio-oceanographic and operative characteristics required to set a large scale grow-out.

The grow-out of taquilla clam seeds is feasible and can yield harvests of small clams as baby clams after one year of culturing. The grow-out of clam seeds could become a new economic activity for artisan fishermen and small *Gracilaria* culturers in southern Chile, specifically in the Región de Los Lagos. The management conditions may be improved, removing the algae from the systems, controlling the levels of sediments and spreading out the clams, with the object of increasing the growth rate and survival of the seeds.

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