Research Article

Spawning induction and embryonic development of the clam *Ameghinomya antiqua* (King, 1832)

**Catalina Lizama[1] , Alejandro Abarca[2] , Luis René Durán[1,3] & Doris Oliva[1,4] **
[1]Centro de Investigación y Gestión de los Recursos Naturales (CIGREN)
Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile
[2]Departamento de Acuicultura, Facultad de Ciencias del Mar, Universidad Católica del Norte
Coquimbo, Chile
[3]Copulhue SpA, Conchas Blancas, Quinchao Island, Chiloé, Chile
[4]Instituto de Biología, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile
Corresponding author: Doris Oliva (doris.oliva@uv.cl)

ABSTRACT. Artificial cultivation increases clams' availability and is an alternative to the extraction from natural banks. The culture of clams requires species-specific research in the different growth stages, and studies on the effects and interactions of culture parameters are essential to obtain and control the proper development of larvae. This paper aims to compare methods to induce spawning, describe the embryonic development, and compare the effect of different culture densities on the yield of "D" larvae of the "taca" clam *Ameghinomya antiqua.* Breeders were collected on the southwest coast of Quinchao Island, Chiloé, Chile. Spawning induction assays were performed comparing different combinations of biological and physical factors. Experiments on the effect of embryonic density in the obtention of "D" larvae were performed, and the embryonic development was described at $11 \pm 1°C$. The spawning inductions were successfully achieved with the addition of food combined with temperature changes, resulting in the liberation of oocytes with a jelly coat with a diameter of 140 µm. Trochophore larvae were observed at 40 h post-fertilization. The percentage of embryos developed showed significant differences when testing cultures with densities of 20, 40, and 60 embryos mL^{-1}. Experiments with 20 embryos mL^{-1} density were the ones that obtained a greater number of developed embryos (50%). These results suggest spawning induction with the addition of food and temperature changes with a density of 20 embryos mL^{-1}. This paper describes the embryonic development and technology development for spawning induction for the first time.

Keywords: *Ameghinomya antiqua*; clam culture; jelly coat; small-scale aquaculture; southern Pacific

INTRODUCTION

In Chilean clam fisheries, *Ameghinomya antiqua* (King, 1832) represents 72.87% of the national landing (SERNAPESCA 2021). The *A. antiqua* fishery has shown a sharp decline over time (Jerez et al. 2006, González & Barahona 2007, Jerez & Figueroa 2008) with 43,761 t in 1988 and 11,288 t in 2020 (SERNAPESCA 2022). Authorized artisanal divers carry out clam extraction from the natural banks.

The Undersecretary of Fisheries (SUBPESCA by its spanish acronym) has implemented measures for the recovery of natural banks and the development and diversification of aquaculture activities in management areas (AMERB) and small-scale aquaculture (APE) with native species (Uriarte 2008). *A. antiqua* has been one of the five selected species to enhance research in the cultivation of native resources.

The Veneriidae, *A. antiqua* (=*Venus antiqua),* has separate sexes with two spawning periods in an annual

_____

cycle (Zagal & Hermosilla 2007) with maximum maturity in austral spring and summer (September and February) (Lozada & Bustos 1984). The minimum legal extraction size is 5.5 cm (Jaramillo et al. 2003, SERNAPESCA 2020). Because of the actual status of the fishery, a two-year ban is in effect in the main extraction area, which accounts for 70.96% of the landing (SUBPESCA 2021).

Studies on the cultivation of *A. antiqua* addressed the development of technology for the mass production of spats and their subsequent fattening in the environment (Bustos & Olavarría 2000, González & Barahona 2007). Currently, there are no commercial cultured clams in Chile; only experimental and pilot cultures with fattening in the natural environment have been carried out (FAO 2016).

The induction to spawning is key in the cultivation of bivalves. Physical stimuli are the most used, among them temperature variation (thermal shock) and biological stimulation using gonadal extracts (Reverol et al. 2004, Helm et al. 2006, Barría et al. 2021). The thermal shock is the less stressful method and induces a successful gametes expulsion (Bustos & Olavarría 2000, Helm et al. 2006, Ng et al. 2009, Rengel et al. 2009, Aranda-Burgos et al. 2014, Castillo-Durán et al. 2015).

Species-specific research is necessary as each species has particularities in embryonic development and in the requirements of culture conditions (Helm et al. 2006, Gosling 2015). Factors affecting bivalve growth in their developmental stages are water column depth, temperature, food, salinity, and culture density (Gosling 2004, 2015, Helm et al. 2006, Liu et al. 2006, 2010, Huo et al. 2017). These issues have been addressed in studies of embryonic, larval, and postlarval development in clams (Reverol et al. 1998, 2004, Liu et al. 2006, 2010, Mouëza et al. 2006, Yan et al. 2006, Rengel et al. 2009, Jagadis 2011, Aranda-Burgos et al. 2014, Contreras-Guzmán et al. 2014, Castillo-Durán et al. 2015, Huo et al. 2017).

The culture density of embryos has been studied in the clam *Mercenaria mercenaria*, showing that at high embryo densities, the development only reaches the morula stage, and abnormal larvae are obtained (Loosanoff & Davis 1963). Helm et al. (2006) mentioned that for many of the commonly cultured oviparous oysters and clams, embryo culture density could be as high as 50 to 80 mL^{-1}, but 20 mL^{-1} is considered more suitable. For *A. antiqua*, Bustos & Olavarría (2000) used an embryo culture density of 40 and 60 mL^{-1}. The pectinid *Chlamys asperrima* embryos can be incubated at densities up to 100 mL^{-1} with an effective yield of D larvae at the end of incubation (O'Connor & Heasman 1995).

Identifying the optimal ranges of factors such as density, temperature, and feeding for each species is critical for successful and efficient production (Loosanoff & Davis 1963, Gruffydd & Beaumont 1970, Liu et al. 2010). On the other hand, studying the effects and interactions between physical, chemical, and biological parameters is essential to obtain and control adequate larval development (Reverol et al. 1998, Castagna 2001).

Based on the above background, this study seeks to describe the embryonic development and obtain the technology for the larval culture of the clam *A. antiqua*, considering methodologies for the induction to spawn and the effect of the embryonic density on the D larvae production.

MATERIALS AND METHODS

Spawning induction of *Ameghinomya antiqua*

Breeders (n = 211; mean length = 45 ± 4.2 mm) were collected during austral spring (September-October) from the southwest coast of Quinchao Island (42°29'S, 73°30'W), Chiloé, Chile.

In the Copulhue SpA hatchery, the clams' maturity was evaluated in a sample before induction by macroscopic analysis of the gonad. Clams were randomly selected, and three clams per sex were used for gonad analysis according to Helm et al. (2006) methodology. An optical microscope (AMSCOPE 40C-200x camera for photographic recording) was used to observe spermatozoa mobility (400x) and the presence of mature oocytes (100x).

Seven methods were sequentially tested for spawning induction using 30 breeders per treatment with three replicates for each one: 1) biological: spermatozoa obtained by gonad stripping were diluted in 500 mL of filtered seawater and added to the trays with the breeders; 2) gradual temperature increase: seawater temperature was increased by 2°C every 30 min from 9 to 23°C using a heater controlled by a thermostat; 3) sudden temperature change: clams were kept in a 9°C tank for 30 min and then changed to a 19°C tank, this sudden change was repeated four times (Bustos & Olavarría 2000); 4) gradual temperature increase plus biological induction: stripped male gonad diluted in 250 mL of filtered seawater was added to the trays. The temperature was increased by 2°C every 30 min until it reached 23°C when an extra 250 mL of diluted spermatozoa were added; 5) dry periods and sudden temperature changes: breeders were kept out of water for 30 min, then immersed at 9°C water and subsequently in 19°C water. This cycle was repeated three times; 6) salinity changes, dry periods, and sudden

Table 1. Assays for the spawning induction of *Ameghinomya antiqua* with biological and physical factors.

Induction methods	Description	Aeration
(1) Biological	500 mL of seawater with spermatozoa at 12°C.	Yes
(2) Gradual temperature increase	Gradual increase from 9 to 23ºC.	Yes
(3) Sudden temperature change	Sudden change from 9 to 19°C in four cycles.	No
(4) Gradual temperature increases plus biological induction	250 mL of sperm maceration + gradual increase from 11 to 23ºC + 250 mL of sperm maceration	Yes
(5) Dry periods and sudden temperature changes	Sudden changes of T° from 9 to 19°C + 30 min dry periods (breeders) between seawater changes.	No
(6) Salinity change, dry periods, and sudden temperature change plus biological induction	One hour in fresh water at 9°C + sudden changes in T° from 9 to 19°C + dry breeders between seawater changes + 250 mL of sperm macerate	Yes, during the time with sperm
(7) Induction with the addition of food and temperature increase	3 L of microalgae + heater for 12 h up to 18-20ºC + without heater with 3 L microalgae for 1 h + cleaning with fresh water + breeders at 11ºC with 3 L of microalgae.	Yes, during the time with heater

temperature changes plus biological induction. Individuals were left for 1 h in fresh water at 9°C; then, three cycles were performed with periods of 30 min dry, warm water, and finally cold seawater. Once the cycles were finished, 250 mL of sperm dilution was added; 7) induction with the addition of food and temperature increase: the breeders were fed with 3 L of microalgae (*Nannochloropsis* sp., *Tisochrysis lutea* and *Chaetoceros muelleri*), without aeration and with a heater controlled by thermostat for 12 h, increasing the temperature to 18-20°C. Then, the heater was cut off, 3 L of microalgae was added, and after 1 h, the breeders were removed, cleaned with fresh water, and put back in seawater at 11°C with 3 L of microalgae and without aeration (Table 1).

Embryonic development in *Ameghinomya antiqua*

In 1 L of seawater filtered at 11 ± 1°C, the newly released oocytes were placed with 3 mL of seawater with spermatozoa to perform fertilization in an oocyte/sperm ratio of 1:5 and an embryonic density of 40 embryos mL^{-1} (Bustos & Olavarría 2000) The eggs and spermatozoa were obtained from spawning induction experiment with the addition of food and temperature increase (7).

Every 15 min during the first 3 h, one aliquot of 1 mL was extracted to control the time of appearance of the different developmental phases. Then the controls were performed every 30 min until the embryos reached the gastrula stage and every 2 h until the larval state D was observed. The samples were observed *in vivo* under an optical microscope. The time limit between embryonic development phases was established as the one in which 50% of the sample achieved a specific phase. The samples were observed in a Sedgwick Rafter chamber under a field optical microscope.

Effect of embryonic density for obtaining D larvae

After fertilization, the embryos were sieved and placed in a 1 L test tube with seawater filtered at 1 μm. Homogeneous samples of 1 mL were counted in a Sedgwick Rafter chamber under an optical microscope (Amscope).

The experimental densities were selected based on the densities used by Bustos & Olavarría (2020) in *A. antiqua* and those suggested in the literature for oysters and clams (Helm et al. 2006). Three treatments were used in the embryonic densities experiment: 20, 40, and 60 embryos mL^{-1}, each with three replicates. The embryos were placed in plastic containers (HDPE) with 10 L of filtered seawater at 14°C, and aeration stones (13×25 mm) were used with low pressure to avoid water stirring.

Then, after 60 h, the homogeneous samples were taken in 1 L. Samples of 1 mL were taken, placed in a Sedgwick Rafter chamber, and observed under an optical microscope. D larvae and embryos were counted, and their length and height were measured. The D larvae obtained were determined as a percentage of the initial number of embryos in the sample to evaluate the effect of different densities.

Data analysis

A Shapiro-Wilks test was performed to assess normality and a Levene test to check homoscedasticity. The effect of embryonic density on the percentage of embryos developed and D larvae obtained was analyzed with an ANOVA, and the differences between the means with Tukey *a posteriori* test. The same test was applied to the D larval length and height. A $P < 0.05$ was used as a significance criterion for all tests. Data were analyzed using Minitab 18 statistical software.

RESULTS

Spawning induction of *Ameghinomya antiqua*

Table 2 shows the results of the induction experiments. In treatment (1) with biological induction (addition of spermatozoa), 90% of the individuals opened their valves with siphons fully extended, and, in some cases, an active displacement was registered. In addition, the expulsion of pseudofeces was observed.

In the induction with the gradual temperature increase method (2), when rising the temperature, the clams closed the valves for the first 10 to 15 min, then reopened them and extended their siphons. In treatment (3) with sudden temperature changes, the breeders remained with their valves closed throughout the experiment.

On the other hand, when the combination of gradual temperature increases and biological induction with stripped male gonad was tested (treatment 4), the clams remained with their siphons extended during the whole period; additionally, pseudofeces were expelled, and the animals moved actively. In the treatment with dry periods and sudden temperature changes (5), the clams remained closed for the first 25 min. Then, in the last 5 min of the cycle, some individuals began to extend their siphons, which was interrupted when the next cycle started. In the last cycle, 95% of the breeders remained with their valves closed for 30 min.

In the treatment (6) with salinity changes, dry periods, and sudden temperature changes plus biological induction, the clams remained with closed valves during the experiment. The food and temperature variation method (7) produced an abundant spawning (Fig. 1) with a success rate of 99% of the induced breeders. Prior to spawning, the individuals presented fully extended siphons without movement nor pseudofeces expulsion. The clams that initiated the expulsion of their gametes generated coordination with the other specimens that began to spawn. That is, an individual expelled his gametes for a short time and then stopped emitting, and another individual began the partial expulsion of his gametes; after the second individual stopped emitting, the first one spawned again, and so on.

Males emitted their gametes with a continuous flow, forming a white ribbon suspended in the water column. The females expulsed the gametes in a continuous grainy white flow that sank to the bottom. Once the gametes were expelled, the water turned completely milky. Hydrated oocytes with a jelly coat had a diameter of 140 ± 50 μm (Fig. 2) and without the jelly coat of 90 ± 20 μm. Spermatozoa had a sickle-shaped form, high motility, and a length of 5 ± 0.5 μm.

Embryonic development

The post-fertilization time and size for each development stage is summarized in Table 3. Successful fertilization was indicated by the presence of the fertilization membrane (Fig. 3a) at 20 min after adding the sperm to the water with oocytes. In oocytes with fertilization membrane, the jelly coat is not visible anymore. The first polar corpuscle was observed at 45 min post-fertilization (pf). At 2 h and 15 min pf, the first mitotic cleavage was observed, dividing the egg unevenly into two cells (Fig. 3b).

The second division was seen at 3 h and 15 min pf, forming a tetrad with a macromer and three micromeres (Fig. 3c). After 4 h and 15 min, the third cleavage occurred, forming eight cells, maintaining a macromer and several irregular micromeres (Fig. 3d). The morula was observed at 6 h and 15 min pf (Fig. 3e), with no sign of a macromer. At 12 h pf, the blastula with the blastopore generated by cellular invagination was formed (Fig. 3f), and after 15 h pf, the rotary blastula was observed (Fig. 3g) with slight movements and spun on its axis (Fig. 3h). After 16 h pf, the gastrula (Fig. 3i) stage began, with cilia on its surface, allowing a rotatory displacement in different directions. Finally, at 40 h pf, the trochophore larval phase was reached (Fig. 3j). This larva had an oval shape, was covered by cilia, and had a long apical flagellum that allowed high mobility and displacement with spins around its axis.

Effect of embryonic density for obtaining D larvae

A one-way ANOVA showed no significant differences between the three density embryo treatments ($F = 0.22$; $P > 0.80$). In the treatment with a density of 20 embryos mL^{-1}, $47.2 \pm 6.7\%$ (average $\pm$ standard error) of the embryos developed to D larvae. In the treatment with 40 embryos mL^{-1}, a mean value of $48.6 \pm 7.2\%$, and finally, in the treatment with the highest density (60 embryos mL^{-1}), a mean of $43.3 \pm 3.1\%$ of embryos turned to D larvae. Figure 4 shows the proportion of D larvae generated at the end of the experiment at a temperature of 14°C.

The mean lengths of the D larvae obtained in the different embryo densities experiments were 121 ± 5 μm for 20 embryos mL^{-1}, 121.6 ± 8.5 μm for 40 embryos mL^{-1}, and 122.6 ± 4.2 μm for 60 embryos mL^{-1}. A one-way ANOVA showed no significant differences between the mean length of D larvae in the three density treatments ($F = 0.71$; $P > 0.49$). The height of D larvae also does not show significant differences between treatments ($F = 0.65$; $P > 0.52$).

Table 2. Results of spawning induction experiments in *Ameghinomya antiqua*.

Types of induction	Observation	Observation time (h)	Spawning
(1) Biological	Extended siphons and expulsion of pseudofeces	3	No spawning
(2) Gradual temperature increase	Extended siphons	3	No spawning
(3) Sudden temperature change	Closed valves	6	No spawning
(4) Gradual temperature increase plus biological induction	Extended siphons and expulsion of pseudofeces	3	No spawning
(5) Dry periods and sudden temperature changes	Closed valves	8	No spawning
(6) Salinity change, dry periods, and sudden temperature change plus biological induction	Closed valves	13	No spawning
(7) Induction with the addition of food and temperature increase	Extended siphons, without displacement and expulsion of pseudofeces	35	Abundant spawning

Figure 1. Spawning of male *Ameghinomya antiqua*.

Figure 2. *Ameghinomya antiqua* oocyte. Oocyte with its jelly coat (JC) at 100x. Scale bar = 50 μm.

DISCUSSION

Among the methodologies described in the literature to achieve the expulsion of gametes, the most used for clams is the thermal shock with a high percentage of spawning (Bustos & Olavarría 2000, Castagna 2001, Helm et al. 2006, Hadley &Whetstone 2007, Da Costa et al. 2008, Contreras-Guzmán et al. 2014). This methodology has been used with previous conditioning in *Mercenaria mercenaria*, *Ruditapes philippinarum*, *Chione cancellata*, *Ameghinomya antiqua*, *Chionista fluctifraga*, and *Pholas orientalis* with temperature differences between 8-9°C and induction periods of 2 to 3 h (Bustos & Olavarría 2000, Castagna 2001, Ng et al. 2009, Rengel et al. 2009, Aranda-Burgos et al. 2014, Castillo-Durán et al. 2015). The gradual temperature increase has been successful for *Katelysia rhytiphora*

and *Mulinia edulis* (Nell et al. 1994, Oliva et al. 2013). In *Tawera elliptica* and *Tivela mactroides* the best results have been achieved with biological induction with gonad extract (Reverol et al. 2004, Barría et al. 2021) and in *Venerupis pullastra* with desiccation (Cerviño 2011). On the other hand, other authors have used a mix of thermal shock, food and gonadal extract, and a combination of thermal shock and desiccation (Hur et al. 2005, Cerviño 2011, Aranda-Burgos et al. 2014, Contreras-Guzmán et al. 2014). They achieved spawning in *Gari solida*, *R. philippinarum*, *Venerupis pullastra*, *Cyclina sinensis*, and *Metrix lusoria*. However, methodology did in this study not have positive results in this study. The breeders of *A. antiqua* had abundant spawning after the stimulus caused by varying the temperature and adding food, a method not previously used in this species. In these experiments,

Table 3. Characterization of the embryonic development of taca clam (*Ameghinomya antiqua*) at 11 ± 1°C.

Stage	Post-fertilization time	Size (μm)
Fertilization membrane	20 min	85 ± 5
Formation of the first polar corpuscle	45 min	85 ± 5
First cleavage	2 h 15 min	100 ± 10
Second cleavage	3 h 15 min	95 ± 5
Third cleavage	4 h 15 min	95 ± 5
Morula	6 h 15 min	95 ± 5
Blastula	12 h	85 ± 5
Rotary blastula	15 h	85 ± 5
Gastrula	16 h	90 ± 5
Trochophore	40 h	90 ± 10
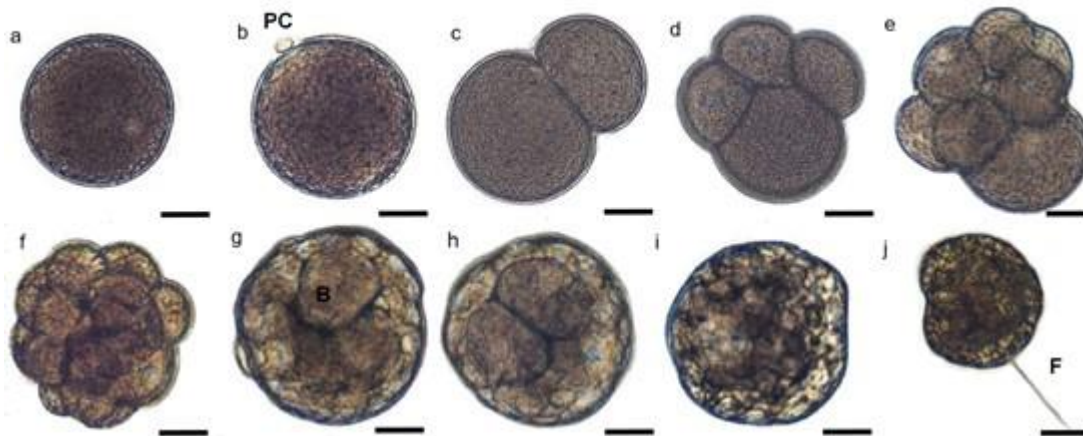
Figure 3. Embryonic development of *Ameghinomya antiqua* at 100x. a) Oocyte with fertilization membrane, b) first polar corpuscle, c) the first cleavage: two cells, d) the second cleavage: four cells, e) third cleavage: eight cells, f) morula, g) blastula, h) rotary blastula, i) gastrula, j) trochophore larvae. PC: polar corpuscle, B: blastopore, F: flagellum. Scale bar = 30 μm.

the clams were induced to spawn in spring without previous conditioning, and 50% of the embryos developed into D larvae in contrast to Bustos & Olavarría (2000), who obtained an 80% with clams conditioned for 45-60 days. In both experiments, the proportion of sperm/ oocyte used was 5:1.

The breeders were not stimulated by inducing spawning with the thermal shock method. These results differed from those of Bustos & Olavarría (2000), who achieved successful spawning with previously conditioned breeders. This difference may be because spawning is dependent on endogenous and exogenous factors (Martínez-Guzmán 2008), such as the initial condition of the breeders (Reverol et al. 2004) and temperature (Vélez et al. 1993). Therefore, Martínez-Guzmán (2008) suggests previous conditioning of the breeders for successful spawning. Many bivalves from cold and warm water habitats require between 4 to 8

weeks of conditioning to reach spawning maturity and disengage from the reproductive periods of the species (Helm et al. 2006). In this study, the breeders were not conditioned, and successful and abundant spawning was achieved. Having two periods of annual spawning, *A. antiqua* may not be conditioned and can be induced to spawn with the gradual increase in temperature and food, thus avoiding the costly hatchery conditioning. Among the exogenous factors, temperature and food availability are key variables in the reproductive cycle of marine bivalves (Freites et al. 2014); in this sense, the coupled effect of these two variables on *A. antiqua* mature breeders led to successful spawning.

The expulsion of gametes in both sexes of *A. antiqua* agreed with the description of Bustos & Olavarría (2000) and Cerviño (2011), and the sex ratio was close to 1:1 as described by Prida et al. (2018). However, the oocytes presented a jelly coat that had not

Table 4. Comparison of oocyte size, developmental time to trochophore, and size of trochophore larvae and D larvae of *Ameghinomya antiqua* with some species of the family Veneridae. pf: post-fertilization; nd: no data.

Species	Total oocyte diameter (µm)	Uncovered oocyte diameter (µm)	Trochophore pf time (h)	Trochophore size (µm)	Temperature (°C)	Reference
Mercenaria mercenaria	163 - 170	70 - 73	nd	nd	22	Loosanoff & Davis (1963)
Tawera elliptica	166.54 ± 9.95	66.92 ±10	56.50'	91.5 ± 7.07	10.7 ± 0.9	Barría et al. (2021)
Venerupis pullastra	65 - 75	nd	13 - 14	80 - 90	19 ± 1	Cerviño (2011)
Ruditapes philippinarum	64.2 ± 3.3	nd	10	nd	22 ± 1	Hur et al. (2005)
Cyclina sinensis	87.4 ± 2.8	nd	10.30'	nd	22 ± 1	Hur et al. (2005)
Metrix lusoria	87.3 ± 3.6	nd	9.30'	nd	26 ± 1	Hur et al. (2005)
Ruditapes decussatus	67.43 ± 3.62	nd	13	nd	20 ± 1	Aranda-Burgos et al. (2014)
Tivela mactroides	60 - 69	nd	10.39'	60 - 69	25 ± 1	Reverol et al. (2004)
Chionista fluctifraga	70.5 ± 1.5	nd	nd	nd	25 ± 2	Castillo-Durán et al. (2015)
Gafrarium tumidum	56 - 63	nd	7	nd	28.5 - 30	Jagadis (2011)
Ameghinomya antiqua (=*Venus antiqua*)	60 - 65	nd	16	70	16	Bustos & Olavarría (2000)
Ameghinomya antiqua	140 ± 50	90 ± 20	40	90 ± 10	11 ± 1	Present study

been described for the species in previous studies (Olavarría et al. 1996, Bustos & Olavarría 2000). The oocytes with the jelly coat have a diameter of 140 ± 50 µm, and without the jelly coat, 90 ± 20 µm, values that are greater than those indicated by Bustos & Olavarría (2000) of 60-65 µm. Within the family Veneridae, species without gelatinous cover have similar oocytes diameters (Table 4). The gelatinous-coated species are *M. mercenaria* (Loosanoff & Davis 1963) and *T. elliptica* (Barría et al. 2021) (Table 4), with diameters similar to those obtained in this study.

The jelly coat is an extracellular structure with different functions surrounding oocytes and embryos (Barría et al. 2021). In female gametes of certain species, it prevents the penetration of more sperm (Cerviño 2011), thus avoiding polyspermy (Gosling 2015).

The embryonic development of *A. antiqua* follows the pattern described for bivalve mollusks, where the first, second, and third division stages are achieved, followed by the state of morula, blastula, rotating blastula, gastrula, and trochophore (Reverol et al. 2004, Hur et al. 2005, Da Costa et al. 2008, Aranda-Burgos et al. 2014, Contreras-Guzmán et al. 2014, Barría et al. 2021). Nevertheless, in *A. antiqua,* the jelly coat disappears after the oocyte is fertilized, unlike *T. elliptica* (Barría et al. 2021), where this layer disappears in the stage of gastrula-trochophore, and *G. solida,* the jelly coat remains during the complete embryonic cycle (Contreras-Guzmán et al. 2014).

The trochophore larval stage in the present study was reached at 40 h pf with a length of 90 ±10 µm, whereas Bustos & Olavarría (2000) describe that the trochophore larva is observed at 16 h pf with a length

of 70 µm at a temperature of 16°C. The development times are similar to those described for *Semele solida* cultured at 22°C and *M. edulis* at 9.8 ± 1.5°C, which reached the trochophore larval stage at 48 and 34 h, respectively (Cisneros & Bautista 2006, Barría et al. 2017). *T. elliptica*, which also has a jelly coat, reaches trochophore larva in a shorter time than *A. antiqua* (Table 4, Barría et al. 2021). However, the length of the larvae is very similar in both species, cultivated under the same temperature conditions. In the case of *T. elliptica*, the experiments were performed at 10.7 ± 0.9°C and in the present study at 11 ± 1°C. In species such as *G. solida*, the trochophore larvae were obtained within 20 to 23 h (Contreras-Guzmán et al. 2014), within 13-14 h in *V. pullastra* (Cerviño 2011), 13 h in *Ruditapes decussatus* (Aranda-Burgos et al. 2014), around 10 h in *R. philippinarum*, *C. sinensis* and *M. lusoria*, and 7 h in *T. mactroides* and *Gafrarium tumidum* (Reverol et al. 2004, Jagadis 2011) (Table 4). The time to reach the stage of trochophore larvae of *A. antiqua* in this study is longer than in the previous ones on the same species or other clams. The temperature at which embryonic development experiments are carried out is relevant (Loosanoff & Davis 1963, Clotteau & Dubé 1993, Castagna 2001, Hadley & Whetstone 2007), and the species mentioned were cultured in temperatures above 19°C. Therefore this could influence the shorter time of embryonic development up to trochophore larva of those species. When performing embryonic and larval development in the case of *M. edulis*, regardless of the season of the year, the time of appearance of the stages decreases at a higher temperature (Barría et al. 2017).

D larvae were obtained in all experimental densities (Fig. 4). According to Helm et al. (2006), the recommen-
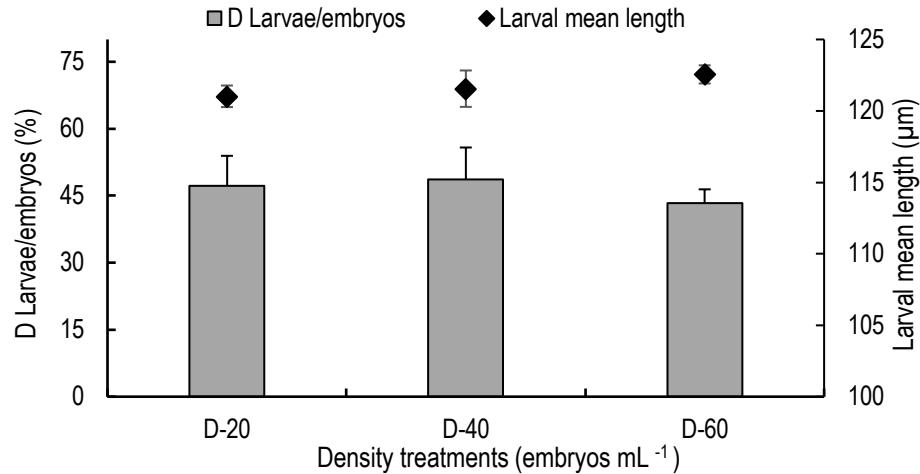
Figure 4. Percentage of D larvae obtained at different embryonic densities (20, 40, and 60 embryos mL^{-1}) and mean larval length ± standard error.

ded densities are 20-40 embryos mL^{-1} for *R. philippinarum* and 15-25 embryos mL^{-1} for *M. mercenaria* and *M. arenaria*. In the case of *M. mercenaria*, high densities were tested and 3000 embryos mL^{-1} did not present developed embryos. However, at 250 embryos mL^{-1}, the complete embryonic cycle was obtained (Loosanoff & Davis 1963). Although there were no differences between the treatments for obtaining D larvae in this study, it is necessary to test higher embryonic densities to obtain a greater number of D Larvae. According to the results, future research should consider the effect of temperature on the embryonic development of *A. antiqua*. In addition, a higher proportion of sperm/oocyte in experiments with higher embryonic densities should be tested to improve the production of D larvae.

ACKNOWLEDGMENTS

REFERENCES

Aranda-Burgos, J.A., Da Costa, F., Nóvoa, S., Ojea, J. & Martínez-Patiño, D. 2014. Embryonic and larval development of *Ruditapes decussatus* (Bivalvia: Veneridae): a study of the shell differentiation process. Journal of Molluscan Studies, 80: 8-16. doi: 10.1093/mollus/eyt044

Barría, C., Abarca, A., Colivoro, J., Celis, A., Couve, P. & Oliva, D. 2017. Efecto de la temperatura y la concentración de espermios en la fecundación, desarrollo embrionario y larval en la almeja *Mulinia edulis* (King, 1832). Congreso de Ciencias del Mar, 23-27 de mayo 2017, PUCV, Valparaíso.

Barría, C., Abarca, A., Colivoro, D., Célis, A., Couve, P., Durán, L.R., et al. 2021. Broodstock conditioning, spawning, embryonic and early larval development of a novel baby clam species for aquaculture in the southeast Pacific: *Tawera elliptica* (Lamarck, 1818). Aquaculture Research, 52: 2759-2771. doi: 10.1111/are.15128

Bustos, E. & Olavarría, E. 2000. Manual: el cultivo de la almeja (*Venus antiqua*). Proyecto CORFO-FONTEC 93-0168. Instituto de Fomento Pesquero, Valparaíso.

Castillo-Durán, A., Hoyos-Chairez, F., Arreola-Lizárraga, J., Martínez-Córdova, L. & Chávez-Villalba, F. 2015. Larval and post-larval growth, spat production and off-bottom cultivation of the smooth venus clam *Chionista fluctifraga*. Aquaculture Research, 47: 2851-2860. doi: 10.1111/are.12735

Castagna, M. 2001. Aquaculture of the hard clam, *Mercenaria mercenaria*. In: Kraeuter, J.N. & Castagna, M. (Eds.). Biology of the hard clam. Elsevier, Amsterdam, pp. 675-697.

Cerviño, A. 2011. Ciclo reproductivo, cultivo en criadero y en el medio natural de la almeja babosa *Venerupis pullastra* (Montagu, 1803). Tesis de Doctorado, Universidad de Santiago de Compostela, Santiago de Compostela.

Cisneros, R. & Bautista, J. 2006. Acondicionamiento y reproducción de la almeja *Semele solida* (Gray, 1828)

en ambiente controlado. V Seminario virtual de las ciencias del mar OANNE. [http://www.oannes.org.pe/upload/201609221530071249515882.pdf]. Reviewed: September 15, 2021.

Clotteau, G. & Dubé, F. 1993. Optimization of fertilization parameters for rearing surf clams (*Spisula solidissima*). Aquaculture, 114: 339-353.

Contreras-Guzmán, R., Puebla-Arce, E., Pacheco-Sánchez, E. & Contreras-Saldaña, F. 2014. Crecimiento y supervivencia de larvas D, pedivéligeras y postlarvas de *Gari solida* (Mollusca: Psammobiidae), provenientes de reproductores acondicionados. Revista de Biología Marina y Oceanografía, 49: 607-614. doi: 10.4067/S0718-19572014000300018

Da Costa, F., Darriba, S. & Martínez-Patino, D. 2008. Embryonic and larval development of *Ensis arcuatus* (Jeffreys, 1865) (Bivalvia: Pharidae). Journal of Molluscan Studies, 74: 103-109. doi: 10.1093/mollus/eym051

Food and Agriculture Organization (FAO). 2016. The state of world fisheries and aquaculture 2016. Contributing to food security and nutrition for all. FAO, Rome.

Freitas, L., Lodeiros, C., Arrieche, D. & Dale, A.W. 2014. Reproductive tactics of marine shellfish species from north-eastern Venezuela: ecophysiological implications. In: Barqueiro, E.R. (Ed.). Spawning, sexual strategies and ecological effects. Nova Science Publishers, New York, pp 73-105.

González, J. & Barahona, N. 2007. Situación de la pesquería del recurso almeja (*Venus antiqua*) en la X Región. Informe Técnico 2, Proyecto BIP #30043687-0. SUBPESCA, Valparaíso.

Gosling, E. 2004. Bivalve molluscs. Fishing News Books, Oxford.

Gosling, E. 2015. Marine bivalve molluscs. John Wiley & Sons, Chichester.

Gruffydd, L.D. & Beaumont, A.R. 1970. Determination of the optimum concentration of eggs and spermatozoa for the production of normal larvae in *Pecten maximus* (Mollusca, Lamellibranchia). Helgolander Wissenschaftliche Meeresuntersuchungen, 20: 486-497.

Hadley, N.H. & Whetstone, J.M. 2007. Hard clam hatchery and nursery production. Southern Regional Aquaculture Center Publication. 4301, Mississippi.

Helm, M., Bourne, N. & Lovatelli, A. 2006. Cultivo de bivalvos en criadero. Un manual práctico. Documento Técnico de Pesca 471. FAO, Rome.

Huo, Z., Guan, H., Rbbani, M., Xiao, Y., Zhang, X., Fan, Z., et al. 2017. Effects of environmental factors on growth, survival, and metamorphosis of geoduck clam (*Panopea japonica* A. Adams, 1850) larvae. Aquaculture Reports, 8: 31-38. doi: 10.1016/j.aqrep.2017.09.001

Hur, Y., Bae, J. & Hur, S. 2005. Comparison of development and larval growth of four venerid clams. Journal of the World Aquaculture Society, 36: 179-187. doi: 10.1111/j.1749-7345.2005.tb00383.x

Jagadis, I. 2011. Spawning, larval development and spat settlement in the Venus clam *Gafrarium tumidum* (Roding, 1798) from southeast coast of India. Indian Journal of Fisheries, 58: 1-5.

Jaramillo, E., Garrido, O., Gallardo, C. & Contreras, H. 2003. Bases biológicas para el ordenamiento de las pesquerías de almejas en la X y XI Regiones. Proyecto FIP 2001-27. Universidad Austral de Chile, Valdivia.

Jerez, G. & Figueroa, M. 2008. Desafíos y perspectivas de la repoblación de moluscos bivalvos en Chile. In: Lovatelli A., Farías, A. & Uriarte, I. (Eds.). Estado actual del cultivo y manejo de moluscos bivalvos y su proyección futura: factores que afectan su sustentabilidad en América Latina. Taller Técnico Regional de la FAO, 20-24 de agosto de 2007, Puerto Montt, Chile. FAO Actas de Pesca y Acuicultura 12. FAO, Rome, pp. 223-235.

Jerez, G., González, J., Tapia, C., Toledo, C., Olguín, A. & Miranda, H. 2006. Ordenamiento de las pesquerías bentónicas de la zona común de pesca de la Comuna de Ancud, X Región. Informe Final, Proyecto FIP 2004-14: 423 pp.

Le, D., Young, T., Alfaro, A., Ragg, N., Hilton, Z., Watts, E. & King, N. 2016. Practical fertilization procedure and embryonic development of the New Zealand geoduck clam (*Panopea zelandica*). Journal of the Marine Biological Association of the United Kingdom, 98: 475-484. doi: 10.1017/S0025315416001685

Liu, W., Gurney-Smith, H., Beerens, A. & Pearce, C. 2010. Effects of stocking density, algal density, and temperature on growth and survival of larvae of the basket cockle, *Clinocardium nuttallii*. Aquaculture, 299: 99-105. doi: 10.1016/j.aquaculture.2009.11.023

Liu, B., Dong, B., Tang, B., Zhang, T. & Xiang, J. 2006. Effect of stocking density on growth, settlement and survival of clam larvae, *Meretrix meretrix*. Aquaculture, 258: 344-349. doi: 10.1016/j.aquaculture.2006.03.047

Loosanoff, V. & Davis, H. 1963. Rearing of bivalve mollusks. Advances in Marine Biology, 1: 1-136. doi: 10.1016/S0065-2881(08)60257-6

Lozada, E. & Bustos, H. 1984. Madurez sexual y fecundidad de *Venus antiqua antiqua* King & Broderip 1835 en la bahía de Ancud (Mollusca: Bivalvia: Veneridae). Revista de Biología Marina, 20: 91-11.

Martínez-Guzmán, G. 2008. Control de la reproducción y producción de semillas de bivalvos en sistemas controlados. In: Lovatelli, A., Farías, A. & Uriarte, I. (Eds.). Estado actual del cultivo y manejo de moluscos bivalvos y su proyección futura: factores que afectan su sustentabilidad en América Latina. Taller Técnico Regional de la FAO. 20-24 de agosto de 2007, Puerto Montt, Chile. FAO Actas de Pesca y Acuicultura 12. FAO, Rome, pp. 267-275.

Mouëza, M., Gros, O. & Frenkiel, L. 2006. Embryonic development and shell differentiation in *Chione cancellata* (Bivalvia, Veneridae): an ultrastructural analysis. Invertebrate Biology, 125: 21-33. doi: 10.1111/j.1744-7410.2006.00036.x

Nell, J., O'Connor, W., Heasman, W. & Goard, L. 1994. Hatchery production for the venerid clam *Katelysia rhytiphora* (Lamy) and the Sydney cockle *Anadara trapezia* (Deshayes). Aquaculture, 119: 149-156. doi: 10.1016/0044-8486(94)90171-6

Ng, B., Tan, A. & Yasin, Z. 2009. Developmental stages, larval and post-larval growth of angelwing clam *Pholas orientalis*. Aquaculture Research, 40: 845-851. doi: 10.1111/j.1365-2109.2009.02177.x

O'Connor, W. & Heasman, M. 1995. Spawning induction and fertilization in the doughboy scallop *Chlamys* (*Mimachlamys*) *asperrima*. Aquaculture, 136: 117-129.

Olavarría, E., Farías, A. & Uriarte, I. 1996. Morfometría y tasas de crecimiento larvario y postlarvario de los bivalvos *Venus antiqua* (King & Broderip, 1835) y *Gari solida* (Gray, 1828) cultivados en laboratorio. Revista de Biología Marina, 31: 107-116.

Oliva, D., Abarca, A., Gutiérrez, R., Celis, Á., Herrera, L. & Pizarro, V. 2013. 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. Revista de Biología Marina y Oceanografía, 48: 37-44. doi: 10.4067/S0718-19572013000100004

Prida, V., Valenzuela, A. & Astorga, M.P. 2018. Evidencia de diferenciación sexual en la almeja *Ameghinomya antiqua* de la costa chilena y su proporción de sexo. Revista de Biología Marina y Oceanografía, 53: 381-385. doi: 10.22370/rbmo.2018.53.3.1361

Rengel, J., Guelmelit, L., Torres, L. & Holuis, C. 2009. Inducción al desove y desarrollo larval del molusco bivalvo *Chione cancellata*. Proceedings of the 62nd Gulf and Caribbean Fisheries Institute, pp. 475-480.

Reverol, Y., Delgado, J., Severeyn, Y. & Severeyn, H. 2004. Embrionary and larval development of the marine clam *Tivela mactroides* (Bivalvia: Veneridae) in Zulia State, Venezuela. Revista de Biología Tropical, 52: 903-909.

Reverol, Y., Severeyn, D., Severeyn, H. & Delgado, J. 1998. Efecto de la temperatura sobre el desarrollo larvario del guacuco, *Tivela mactroides*. Boletín del Centro de Investigaciones Biológicas, 32: 167-177.

Servicio Nacional de Pesca (SERNAPESCA). 2020. Anuario estadístico de pesca y acuicultura 2018. SERNAPESCA, Valparaíso. [http://www.sernapesca.cl/informacion-utilidad/anuarios-estadisticos-de-pesca-y-acuicultura]. Reviewed: September 15, 2021.

Servicio Nacional de Pesca (SERNAPESCA). 2021. Anuario estadístico de pesca y acuicultura 2020. SERNAPESCA, Valparaíso. [http://www.sernapesca.cl/informacion-utilidad/anuarios-estadisticos-de-pesca-y-acuicultura]. Reviewed: September 15, 2021.

Servicio Nacional de Pesca (SERNAPESCA). 2022. Almeja. SERNAPESCA, Valparaíso. [http://www.sernapesca.cl/medidas/almeja]. Reviewed: April, 2022.

Subsecretaría de Pesca (SUBPESCA). 2021. Veda extractiva para recursos del plan de manejo de las pesquerías bentónicas en la bahía de Ancud. SUBPESCA, Valparaíso. [https://www.subpesca.cl/portal/616/articles-106859_documento.pdf]. Reviewed: September 21, 2021.

Uriarte, I. 2008. Estado actual del cultivo de moluscos bivalvos en Chile. In: Lovatelli, A., Farías, A. & Uriarte, I. (Eds.). Estado actual del cultivo y manejo de moluscos bivalvos y su proyección futura: factores que afectan su sustentabilidad en América Latina. Taller Técnico Regional de la FAO. 20-24 de agosto de 2007, Puerto Montt, Chile. FAO Actas de Pesca y Acuicultura 12. FAO, Rome, pp. 61-75.

Vélez, A., Alifa, E. & Freites, L. 1993. Inducción de la reproducción en la vieira *Euvola* (*Pecten*) *ziczac* (Mollusca: Bivalvia). Maduración y desove. Caribbean Journal of Science, 29: 209-213.

Yan, X., Zhang, G. & Yang, F. 2006. Effects of diet, stocking density, and environmental factors on growth, survival, and metamorphosis of Manila clam *Ruditapes philippinarum* larvae. Aquaculture, 253: 350-358. doi: 10.1016/j.aquaculture.2005.07.030

Zagal, C. & Hermosilla, C. 2007. Guía de invertebrados marinos del sur de Chile. Editorial Fantástico Sur, Punta Arenas.