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First description of growth, development and rearing of the sandy clam *Chione cortezi* (Bivalvia, Veneridae) (Carpenter, 1864)

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Abstract. Bivalve molluscs support important fisheries worldwide. In Baja California (BC), Mexico, a state with an extended coastline both in the Pacific and in the Gulf of California, fishing and aquaculture are important activities. Official records indicate that in 2015 the wild fishery contributed 1430 t of bivalves. Among endemic clams species exploited for human consumption in the Gulf of California, it´s found the sandy clam *Chione cortezi* (Carpenter, 1864). The growing demand has led to overfishing of the species, which makes it vulnerable and has put it at risk of disappearing from its natural habitat. The objectives of this study were to describe the external morphology, growth rate, and aspect ratio of larvae through juveniles of *C. cortezi*, under semi-commercial scale laboratory conditions. Culture yielded 5.70 million (M) of D-stage veliger larvae with a mean length (L) (± standard error) of 93.1 ± 0.5 µm and height (H) of 70.8 ± 0.6, 24 h post-fertilization (PF). Pediveligers (H = 235.4 ± 1.5 µm, L = 223.8 ±1.4) were observed after 9 days. Metamorphosis was epinephrine-induced on day 11, and postlarvae reached 2 mm by day 71 (H = 2391.0 ± 88.3 µm, L = 2164.0 ± 78.6). Out of a total production of 2.20 M spat (38.60% survival), 1.5 M were donated to producers for growout in San Felipe. Results of growth rates and development times are compared to those of other venerid clams. This study provides information on the biology of the species and demonstrates the feasibility of *C. cortezi* culture at a scale that can supply the aquaculture industry and lead to development of productive, sustainable fisheries in BC.

Keywords: *Chione cortezi* culture, endemic bivalve, Gulf of California, larval sandy clam, development, spat production

Resumen. Primera descripción del crecimiento, desarrollo y cultivo de la almeja arenera *Chione cortezi* (Bivalvia, Veneridae) (Carpenter, 1864). Los moluscos bivalvos son parte importante de la producción pesquera a nivel mundial. En Baja California (BC), México, un estado con una gran extensión costera tanto en el Pacífico como en el Golfo de California, la pesca y la acuacultura son actividades importantes. Los registros estatales indican que en el año 2015 las pesquerías aportaron 1430 toneladas de bivalvos. Entre las especies endémicas de almejas que se explotan en el Golfo de California para consumo humano, se encuentra la almeja arenera *Chione cortezi* (Carpenter, 1864). La demanda creciente por ésta almeja ha llevado a la sobrepesca de la especie, lo que la vuelve vulnerable y la ha puesto en riesgo de desaparecer de su hábitat. Los objetivos de éste estudio fueron describir la morfología externa, la tasa de crecimiento y, la proporción entre larvas y juveniles de *C. cortezi*, bajo condiciones de laboratorio a escala semi-comercial. El rendimiento en cultivo fue de 5.70 millones (M) de larvas D, con una longitud (L) media (± error estándar) de 93.1 ± 0.5 µm y una altura (H) de 70.8 ± 0.6, 24 h postfertilización (PF). Larvas pedivéligers (H = 235.4 ± 1.5 µm, L = 223.8 ±1.4) se observaron 9 días después. La metamorfosis larval fue inducida con epinefrina en el día 11 y, las postlarvas alcanzaron los 2 mm en el día 71 (H = 2391.0 ± 88.3 µm, L = 2164.0 ±

78.6). Del total de los 2.20 M de semillas producidas (sobrevivencia del 38.60%), 1.5 M fueron donadas a productores de San Felipe para que las crecieran en campo. Los resultados tanto de la tasa como del tiempo de crecimiento, son comparables con los reportados para otras almejas de la familia Veneridae. Este estudio aporta información sobre la biología de la especie y demuestra la viabilidad de cultivar *C. cortezi* a una escala que puede abastecer a la industria acuícola y, permite el desarrollo de pesquerías productivas y sostenibles en BC.

Palabras clave: Cultivo de *Chione cortezi*, bivalvos endémicos, larvas de almeja arenera del Golfo de California, desarrollo larval, producción de juveniles

Introduction

The family Veneridae (Bivalvia) is distributed worldwide, and comprises several genera, including *Chione*, that are important constituents of various benthic communities in the intertidal zone of semi-arid climates, where organisms inhabit fine sand sediment or mud (Moore & Lopez 1969; Benet 1996). The genus *Chione* is represented by at least three Atlantic species, and a minimum of six species in the eastern Pacific, where it is characterized by high diversity (Roopnarine, 2000). The sandy clam, *Chione cortezi*, one of the Pacific species (Carpenter, 1864), is an intertidal bivalve mollusc abundant in the northern Gulf of California (Schöne *et al*. 2002). Its distribution is restricted to the Northern Gulf of California, Mexico, and the delta of the Colorado River, USA (Villarreal-Chávez *et al.* 1999). For decades it was confused with *Chione fluctifraga* until Keen (1971) identified the two known species.

Chione cortezi, an important native constituent of this regional ecosystem, has been used in developing an environmental model to standardize baseline conditions prior to extensive human impacts in the Colorado River watershed (Schöne *et al*. 2003). Its daily growth rate has also been used to model the effects of temperature variation and tidal emergence, using oxygen isotopic profiles in the northern Gulf of California (Goodwin *et al.* 2001). Finally, *C. cortezi* was used as a bioindicator to determine the degree of contamination by organochlorinated pesticides and polychlorinated biphenyls in the Valley of Mexicali and Upper Gulf of California (Gutiérrez-Galindo *et al.* 1988). In addition to the ecological services of *C. cortezi*, the species supports an important fishery resource in Baja California (BC), Mexico.

In 2012, clams were ranked 13 in terms of national fisheries production in Mexico (CONAPESCA, 2014). In BC, a state with an extended coastline (1493 km), fishing and aquaculture activities are economically important. The State Fisheries Department SEPESCA-BC, reports landings of a total of 1430.1 t of bivalve molluscs, including *Chione*. The first records of a *Chione spp.* fishery in BC date to 1998, when 518.2 t were harvested. By 1999, this fishery reached its maximum level (672.6 t) and thereafter, it started to decline until the last report in 2008, which indicated an 85.5% reduction in the fishery, thus yielding only 97.8 t (CONAPESCA, 2014). There are no further official records about this fishery since that year, but regional producers estimate their catch at 55.0 t in 2014. According to the previous numbers, *Chione* species are clearly being overfished and their natural reserves are at risk of disappearing due to a growing market demand and possibly, to environmental modifications. Despite this high demand, there are currently no aquaculture options for *C. cortez*i. The only existing culture alternative for *Chione* species is the smooth venus clam, *C. fluctifraga*, that inhabits the coast of southern California, and the BC Peninsula, including the Gulf of California (Fischer *et al*. 1995). Castillo-Durán *et al.* (2016) reported that spat production of *C. fluctifraga* achieved high field survival and resistance to local climate conditions in Bahía San Jorge, Sonora, Mexico. Despite the availability of cultured *C. fluctifraga* in BC, its production decreased 94% from 2008 to 2013 (from 5.0 to 0.3 t) (CONAPESCA, 2014), yet the *C. cortezi* fishery has not stopped.

Little is known to date about the biology, development, and aquaculture methods to produce juveniles of *C. cortezi*. Therefore, the objectives of the present study are to describe the larval and postlarval rearing of this sandy clam, and to document an effective juvenile (spat) production method for this important native clam species. This will contribute to the development of an efficient aquaculture method as an alternative to prevent its overfishing and possible extinction in BC, Mexico.

Materials and Methods

Broodstock collection and maintenance: A total of 80 adult organisms of *Chione cortezi* with a mean (±Standard Deviation) shell length of 47.0 ± 1.2 mm, width of 51.8 ± 1.5 mm, high of 28.9 ± 0.6 mm, and weight of 53.15 ± 3.8 g, were collected in

September 2015 from a fishing site in San Felipe (30°57'11.10"N, 114°45'7.33"O), Baja California, Mexico. Clams were transported to Instituto de Investigaciones Oceanológicas at Universidad Autónoma de Baja California, and placed in a rectangular 1000 L flat-bottom tank with ultraviolet sterile filtered seawater (SFS) at 20°C ± 1°C and salinity of 33, supplied with continuous air flow. Tank temperature was increased 1°C every two days with submersible heaters (Aqua Heat Titanium Heater 300 Watt) until the SFS reached 24 ± 1°C. For 5 weeks the tank temperature remained constant. Since clams arrived to the laboratory and until its spawning induction (7 weeks after), organisms were fed with a 1:1 cell volume mix of *Tisochrysis lutea* (clone TISO) and *Chaetoceros calcitrans*, at 3% body dry weight per day. Every two days the water was changed with pre-heated SFW to avoid temperature stress in the clams.

Spawning induction and larval production: Spawning was induced by temperature cycling of cool/warm (20°C/30°C) seawater. Once the clams started to release gametes, organisms were separated in individual 1 L recipients. Immediately afterwards, a sample of the gametes was observed under the 10X optical Nikon microscope to determine if it was a female or male clam. Once the clams were sexed, the gametes of 3 clams of the same sex were mixed in 5 L of SFS. A 100 µL sample of either female or male gametes was counted in a Neubauer Chamber to estimate the total amount of gametes contained in each mixture. After knowing the total number of oocytes, the necessary milliliters of the sperm mixture were determined to carry out the fertilization in a sperm:egg ratio of 5000:1.

Oocytes of 3 females were fertilized with sperm pooled from 3 males were placed in a 20 L tank, with SFS at 24°C and salinity of 33, and followed FAO generic protocols for the culture of bivalve molluscs (Helm & Bourne, 2006). Larvae were cultured in 2000 L conical tanks 24 h post-fertilization (PF) at 24°C ± 2, salinity of 33, and a stocking density of 5 larvae mL^{-1}. On day 11, pediveliger larvae were exposed to epinephrine (10^{-5} M, SIGMA) for 4 h to induce settlement/metamorphosis following methods of García-Lavandeira *et al*. (2005). Briefly, pediveliger larvae were collected and placed in 74 x 50 x 10 cm fiberglass containers with a 100 µm bottom mesh, filled with a 10^{-5} M epinephrine solution; after 4 hours of exposition, larvae were carefully rinsed by triplicate with SFS at 24°C ± 2. Larvae were fed with a 1:1 cell volume mix of *Tisochrysis lutea* (clone TISO) and *Chaetoceros calcitrans*, with a total algal concentration of 15000 cells mL^{-1}, and concentration of 50000 cells mL^{-1} at the beginning and end of development, respectively. Seawater was completely replaced every two days with SFS. Larval measurements were carried out at the time of water changes and all measurements are here reported as the mean ± standard error, SE, of 30 organisms; survival was evaluated once a week by counting, i.e., as the average percentage of 3 samples of 1 mL of surviving larvae at each observation interval. All measurements were made from photomicrographs taken from 30 organisms with a Nikon microscope, and analyzed using AxioVision 40 V 4.7.2.0 software. The size of the eggs was determined as the maximum diameter. The shell height (H) and length (L) were measured for D-stage veligers, umboned larvae, and pediveligers. Data were analyzed using the D'Agostino-Pearson normality test (omnibus K2) with a confidence limit of 95%. Measurement data were fitted by linear regressions (95% confidence intervals shown) using a GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, California, USA).

Postlarval rearing: Postlarvae were reared in 74 x 50 x 10 cm fiberglass containers with a 190 µm bottom mesh, at the same temperature and salinity as above. Postlarvae were fed with the same algal mixture but starting at 50000 cells mL^{-1} at the beginning of development and ending at 180000 cells mL^{-1}, with algae supplied twice a day. Once a week, 60-120 postlarvae were measured. Measurements included the shell L and H of 30 individuals and their analysis was conducted as indicated previously. Both wet weight (WW) and survival rate were calculated as the average percentage of postlarvae present in 3 samples of 0.1 g. Wet weight was determined only for 2, 217, 296 postlarvae individuals, and sampling for this purpose was conducted only until it was deemed safe to handle them (day 29 PF).

Results

C. cortezi oocytes showed a mean size of 64.8 µm ± 0.4 SE. After fertilization, a total of 5.7 million fertilized eggs were obtained. Fifteen minutes PF, first cleavage was observed in 25% of the embryos (Fig. 1a). Veliger larvae appeared within 15 h PF, and after 24 h, 100% of D-stage veliger larvae (mean L = 93.1 µm ± 0.5, and H = 70.8 µm ± 0.6) were obtained (Fig. 1b). Umboned larvae were observed after 5 days of culture (Fig. 1c) when they

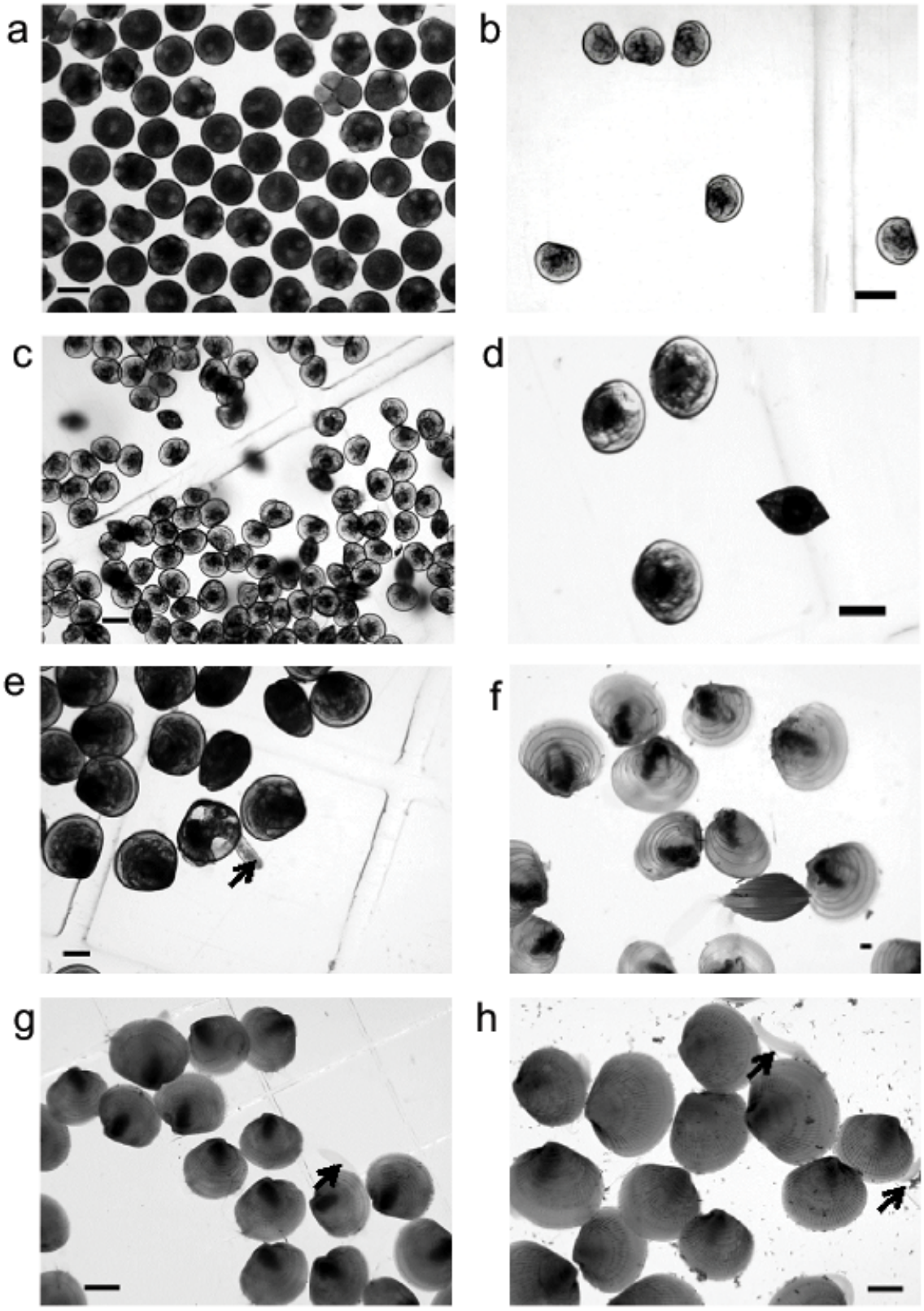

Figure 1. Development stages of the sandy clam, *Chione cortezi*. **a.** Fertilized oocytes after 15 min post-fertilization (PF). **b.** D-stage veliger larvae 24 h PF. **c.** Umboned larvae 5 days PF. **d.** Pediveliger larvae 9 days PF. **e.** Larvae 2 days post-settlement (day 13 PF). **f.** Postlarvae at 28 days PF. **g, h.** Postlarvae at 57 (**g**) and 71 (**h**) days PF. Scale bars: **a** = 50 µm; **b**, **c**, **d**, **e**, **f** = 100 µm; **g**, **h** = 1 mm. Arrows indicate the clam foot.

attained a mean H of 163.4 µm ± 2.6, and L of 140.6 µm ± 2.2. Pediveliger larvae (mean L = 223.8 µm ± 1.4, mean H = 235.4 µm ± 1.5) were observed after 9 days (Fig. 1d). Until this day, cumulative larval survival was 84.0% (Fig. 2). Due the larval color, neither the eye spot nor any other distinctive feature was observed when they were about to set.

Two days after treatment with epinephrine, larvae continued their feed consumption and under the stereoscope, shells looked nearly closed without detritus around them and clams´ foot moved constantly. According to these observations postlarvae appeared healthy and active, and they continued growing to a L of 249.6 µm ± 1.6, and H of 258.4 µm ± 1.8. At this time, approximately 44% of larvae still retained their velum and had thus not yet metamorphosed (Fig. 1e). Due to the concentration of epinephrine used, and since no controls were available, it is not clear whether settlement was induced by this compound. Five days after epinephrine treatment, postlarvae had experienced a cumulative survival of 44.0% (Fig. 2). After 28 days PF, postlarvae showed translucent shells (Fig. 1f), an average L of 682.5 µm ± 10.3, and H of 734.7 µm ± 10.9; survival was 43.0%, and mean WW of 1.03 g ± 0.2 (reported as the mean and SE of 2217296 individuals). It is important to mention that before this day, handling of settled postlarvae was avoided. At day 57 PF shells appeared thicker and showed features characteristic of the *C. cortezi* species (Fig. 1g); at this time, postlarval survival was 37%, mean WW was 2.99 g ± 0.12, mean L was 1622.0 µm ± 26.3, and mean H 1812.0 µm ± 31.3. *Chione cortezi* postlarvae reached 2 mm after 70 days in culture, and cumulative spat survival was 37% from gamete fertilization. By day 71 PF, postlarvae reached an average L of 2164.0 µm ± 78.6, H of 2391.0 µm ± 88.3 (Fig. 1h), a survival of 36%, and their WW averaged 4.74 g ± 0.10 (Fig. 2).

Growth in terms of both length and height throughout development is shown in Figure 3. Larval growth rate based on length measurements was thus calculated as 15.32 µm day^{-1} from the linear equation (coefficient of determination, R^2 = 0.9504, significant at P = 0.0002). Larval growth rate in H was 18.35 µm day^{-1} (R^2 = 0.9702, significant at P < 0.0001). Postlarval growth rates were 30.69 µm day^{-1} in L (R^2 = 0.9800, P < 0.0001), and 34.09 µm day^{-1} in H (R^2 = 0.9792, P < 0.0001). After 71 days PF, a total production of 2.10 million spat was achieved (final survival = 37% of the initial number of fertilized eggs).

Juvenile clams produced were donated to growers from San Felipe, BC, Mexico. Before transport to the growout site (ground transportation in coolers lasted 6 h), clams were exposed for 7 days to a gradual reduction in temperature and acclimated for 3 days in laboratory tanks at the temperature (18°C ± 1) reported in the growout area.

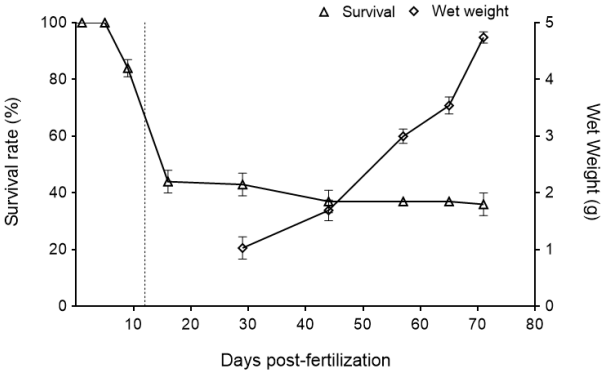

Figure 2. Percent survival rate and wet weight of *Chione cortezi* larvae and postlarvae cultured in the laboratory. Survival rate of both larvae and postlarvae was calculated from day 1 post-fertilization (PF), until day 71 PF. Dashed vertical line indicate the day of metamorphosis induction with epinephrine. Values represent the mean ± standard error, n = 60, 120 per sample; alpha = 0.05.

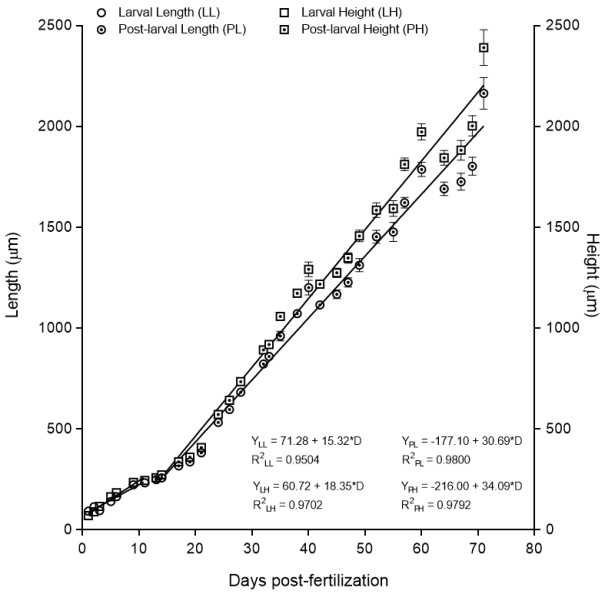

Figure 3. Shell growth rate during *Chione cortezi* development. Data fitted by linear regressions for both the length (left Y axis) and the height (right Y axis) of the larvae (day 1 to 11 PF) and postlarvae (day 13 to 71 PF) of the sandy clam. Final equations and R^2 shown in the right, lower corner of the graph. Data represent the mean ± standard error of the mean, n = 60, 120; P < 0.0001.

Discussion

Venerid clams are of significance due their ecological and commercial importance, as well as density and distribution patterns. The early development of venerid clams is relatively well studied, e.g., in the Sea of Japan (Evseev *et al.* 2001; Hur *et al.* 2005; Nakamura *et al.* 2010), Mediterranean Sea (Ramón *et al.* 2005; Galimany *et al.* 2015), New Zealand (Kent *et al.* 1999; Gribben *et al.* 2002), Brazil (Borzone *et al.* 2001; Oliveira *et al.* 2016), and in the Gulf of California (Villarreal-Chávez *et al.* 1999; Garcia-Cuellar et al. 2004). However, there are relatively few reports about the development of *Chione* spp. describing the early larval stages, metamorphosis, larval settling, and juvenile growth of these clams.

Despite the development of current electron and scanning microscopy and molecular techniques to distinguish larval stages of different bivalve larvae, their identification is usually based upon external morphology such as color, length, aspect ratio, shape and hinges of anterior and posterior ends, and umbo shape and position (Lutz *et al.* 1982; Quayle & Newkirk 1989; Hendriks *et al.* 2005). Knowledge of the morphological differences in larval and postlarval development of venerid clams provides important information to understand bivalve marine life cycles, recruitment, seed collection, and spat production for aquaculture. The current study describes larval and postlarval characteristics of *Chione cortezi*, an important venerid clam in the Gulf of California, and summarizes what is known of larval development in the family Veneridae worldwide. The external morphology, growth rate, and height to length ratio of *C. cortezi*, are reported under semi-commercial scale laboratory conditions.

Although venerid clams are widely distributed, available studies of early development in culture of this bivalve family from 1967 to date, have been conducted at temperatures ranging widely from 20 to 32°C, and salinities between 20 and 34. It is well known that differences in both temperature and salinity generally lead to considerable differences in development time and size (Davis & Calabrese 1964; Gireesh & Gopinathan 2004).Typically, however, some of the reported ranges of physicochemical culture conditions are relatively narrow and may be similar to the environmental conditions to which the clams are exposed in their BC habitat over a year. This may explain the similarities that we found between *C. cortezi* and other venerid clams.

Similarities were observed between the sizes of gametes, larvae, and postlarvae of *C. cortezi* and those of other venerids, as well as in the duration of each development stage under optimum environmental conditions, as presented in Table I. A comparison with other venerid species, indicates that the mean diameter (64.8 μm ± 0.4) of *C. cortezi* oocytes spawned in seawater is similar to that of *Anomalocardia brasiliana* (Mouëza *et al.* 1999), *Anadara trapezia*, *Katelysia rhytiphora* (Nell *et al.* 1994), *Ruditapes philippinarum* (Hur *et al.* 2005), *Tapes dorsatus* (Nell *et al.* 1995), *Mercenaria mercenaria* and *Pitar morrhuanus* (Goodsell *et al.* 1992). Somewhat larger oocytes (by 1.3x) were reported in *Cyclina sinensis* and *Meretrix lusoria* (Table I). Measurements of the spermatozoa of *C. cortezi* were not determined in the present study, but microscopic observation indicated that sperm were very active and presented a short head and a long flagellum (picture not shown), in agreement with descriptions for *A. brasiliana* (Mouëza *et al.* 1999).

In addition to the above similarities between the mean oocyte diameter of *C. cortezi* and other cultured venerid clams, the size of D-shaped larvae of *C. cortezi* larvae were also comparable to those of a number of other venerid species, although smaller than those of *C. sinensis* and *M. lusoria*, the two species that were also characterized by larger oocytes (Table I). In the present study 100% of D-larvae after 24 h of culture exhibited a mean size of 93.1 x 70.8 μm (L x H). On day 1 PF no significant mortality was determined in larval cultures, which differs greatly from the 42.2% reported for *A. brasiliana* (Oliveira *et al.* 2016) and the high mortality (>95%) reported in *R. largillierti* (Kent et al. 1999). Despite differences in culture temperature and salinity, the same development time is reported for *K. rhytiphora* D-larvae (Nell *et al.* 1994), for *Chione stutchburyi*, (Stephenson & Chanley 1979), and for *A. brasiliana* (Mouëza *et al.* 1999; Oliveira *et al.* 2016). Development time and size are similar for straight-hinged (D) larvae of *C. cortezi*, *Chionista fluctifraga* (Castillo-Durán *et al.* 2016), and *Marcia opima* (Muthiah *et al.* 2002). Straight-hinged larvae with different development times although similar sizes were observed in *A. trapezia* (Nell *et al.* 1994), and *R. largillierti* (Kent *et al.* 1999). Smaller D-stage larvae than *C. cortezi* were reported in *C. stutchburyi* (Hur *et al*. 2005) and *A. brasiliana* (Table I). Development times to D-stage were comparable (1-2 days), however, among most

Table I. Comparison of *Chione cortezi* development stages with other venerid species. Size is reported in µm, mean ± standard error in parenthesis () and sample size *n*, or standard deviation in parenthesis with asterisk ()*; ranges are given when the error term was not reported; L = Length; H = Height; Days PF = Days Post-Fertilization; % Surv.= percent survival based on the initial number of fertilized oocytes; T°C=Temperature. Shell growth rate is reported from straight-hinged to pediveliger larval stage (based on fitted linear or exponential functions to the data). Microalgal diet in cell volume mix (a) or in equal dry weight basis (b); Pl = *Pavlova lutheri*; TISO = *Tisochrysis lutea* (clone TISO); Cc = *Chaetoceros calcitrans*; Nsp = *Nannochloris* sp.; No = *Nannochloropsis oculata*; Tt = *Tetraselmis tetratele*; Psp = *Platymonas* sp.; Ch = *Chlorella* sp.; Ml = *Monochrysis lutheri*; Ts = *Tetraselmis suecica*; Cg = *Chaetoceros gracilis*; Csp = *Chaetoceros* sp. When neither temperature nor salinity is specified by the authors, the location of the research site is provided. No reported values are indicated by a hyphen. Note that oocyte size refers to spawned, unfertilized oocytes.

Veneridae species/ common name	Oocyte size	Straight-hinged		Pediveliger			Shell growth rate µmday^{-1}	T °C	Salinity	Microalgal Diet	Reference
		Size	Days PF	Size	Days PF	% Surv.					
Anadara trapezia Sydney Cockle	62.6 (2.6)*	L 92.0 (3.3)* H 69.5 (4.2)*	2	L 220 H -	14-16	64	-	24 (1)	34	Pl:TISO:Cc (b)	Nell *et al.* 1994
Anomalocardia brasiliana Carib pointed venus	60.0	L 71.9 (24.7)* H 67.3 (15.7)*	1	L 183.2 (20.8)* H 157.9 (18.5)*	10	100	-	25; 27 (2)	30-34	Nsp:TISO:Pl (a); TISO:Cc:No: Tt (a)	Mouëza *et al.* 1999; Oliveira *et al.* 2016
Chione cancellata Cross-barred venus	-	L 125; 87-125 H 108;72-ll9 *n*=5	1	L 240;150-196 H 224;128-l87 *n*=5	8-11	-	L 1.0	23-25; ≈25	20	Psp:Ch (a); TISO:Ml (a)	D'Asaro 1967; LaBarbera and Chanley 1970
Chione cortezi Sandy clam	64.8 (0.4) n=30	L 93.1 (0.5) H 70.8 (0.6) *n*=30	1	L 223.8 (1.6) H 235.4 (1.5) *n*=30	9-11	84	L 15.3 H 18.4	24	33	TISO:Cc (a)	Present work
Chione stutchburyi Stutchbury's venus	-	L 61.6 (1.7) H NR *n*=130	2	L 175-215 H 158-197	-	-	-	23-31	33	Ts:Pl:TISO:Cc (a)	Stephenson and Chanley 1979
Chionista fluctifraga Smooth venus	70.5 (1.5)*	L 80.3 (1.2)* H 70.3 (1.3)*	<1	L 234.5 (14.6)* H 215 (83)*	8-13	~80	H 30.0	24-26	36	TISO:Cc (a)	Castillo-Durán *et al.* 2016
Cyclina sinensis Oriental cyclina	87.4 (2.8)*	L 141.9 (13.8)* H -	6	L 186.7 (9.3)* H -	17	-	-	22	-	Pl:TISO:Cg (a)	Hur *et al.* 2005
Katelysia rhytiphora Sand cockle	66.3 (1.0)*	L 97.1 (3.7)* H 82.0 (2.0)*	1	L 195 H -	11	42	-	20	34	Pl:TISO:Cc (b)	Nell *et al.* 1994
Mactra veneriformis now *M. quadrangularis* Venus mactra	52.8 (2.6)*	L 94.1 (11.7)* H -	5	L 216.0 (12.3)* H -	14	-	-	22	-	Pl:TISO:Cg (a)	Hur *et al.* 2005
Marcia opima Fertile venus	-	L 87 (2.7)* H 77 (6.8)*	1	L 214.5 (13.7)* H 198 (13.5)*	8-11	-	L 1.0	28-31	34-36	TISO:Csp (a)	Muthiah *et al*. 2002
Mercenaria mercenaria Northern quahog	68.0 (3.7)*	-	-	-	-	-	-	Milford, CT, USA		-	Goodsell *et al.* 1992;

Table I. continued.

Veneridae species/ common name	Oocyte size	Straight-hinged		Pediveliger			Shell growth rate µmday^{-1}	T °C	Salinity	Microalgal Diet	Reference
		Size	Days PF	Size	Days PF	% Surv.					
Meretrix lusoria Japanese hard clam	87.3 (3.6)*	L 154.1 (15.3)* H -	2	L 208.1 (13.6)* H -	7	-	-	26	-	Pl:TISO:Cg (a)	Hur *et al.* 2005
Meretrix meretrix Asiatic hard clam	-	L 116.4 H 91.3	<1	L 161.9 H 141.7	6-8	-	H 1.0	30.5 - 32.5	33-34.1	TISO	Narasimham *et al*. 1988
Pitar morrhuanus False quahog	69.0 (2.3)*	-	-	-	-	-	-	Wachap., VA, USA		-	Goodsell *et al.* 1992
Ruditapes largillierti Oblong venus	-	L 85.3 (4.7) H – *n*= 10-40	2	L 200.3 (7.3) H N- *n*=10-40	11-19	<3	-	20	-	Pl:TISO (a)	Kent *et al.* 1999
Ruditapes philippinarum Japanese carpet shell	64.2 (3.3)*	L 104.6 (12.1)* H –	5	L 208.6 (8.9)* H –	13	-	-	22	-	Pl:TISO:Cg (a)	Hur *et al.* 2005
Tapes dorsatus now *T. conspersus* Turgid Tapes	67.7 (3.6)*	L 97.1 (3.7)* H 82.0 (2.0)*	2	L - H 195.0	11-12	-	-	23	-	Pl: TISO:Cc (a)	Nell *et al.* 1995

venerid species listed in Table I, except for *C. sinensis* and *R. philippinarum* (5-6 days).

Studies of other venerid clams indicated that after 3 to 7 days PF, umboned larvae occurred in cultures characterized by lower survival rates (e.g., 20% for *A. brasiliana* (Oliveira *et al.* 2016) compared to the 100% here reported for *C. cortezi*. Umboned veliger larvae of *C. cortezi* were observed at day 5 PF with a L of 140.6 ± 2.3 µm and H of a 163.4 ± 2.6. Similar results in terms of both development time and size were reported for *C. fluctifraga* (salinity of 36), *R. philippinarum*, and *M. veneriformis* (Hur *et al.* 2005; Castillo-Durán *et al.* 2016). Similar sizes were reported for *C. stutchburyi* umboned larvae but specific development times were not reported (Stephenson & Chanley 1979). Umboned larvae of *C. cancellata*, *Anomalocardia brasiliana*, and *M. opima* exhibited a similar development time than umboned *C. cortezi* but differed in their reported sizes (D'Asaro 1967; LaBarbera & Chanley 1970; Muthiah *et al.* 2002; Oliveira *et al.* 2016). Conversely, *M. lusoria* and *C. sinensis* attained similar sizes at different times (Hur *et al.* 2005). Also, the presence of umboned larvae reported for *K. rhytiphora* and *T. dorsatus* after a comparable culture time (4 days PF), were similar to those described for *C. cortezi* but larval sizes were not reported in these prior studies (Nell *et al.* 1994, 1995).

In umboned larvae, the foot of venerid clams emerges occasionally from the shell but is not functional as in pediveligers, in which it becomes fully functional after 7 to 20 days in culture. This development stage in *C. cortezi* occurs after 9 days PF, which coincides in time and size with *C. cancellata*, *C. fluctifraga*, *M. opima*, and *M. lusoria* (reviewed in Table I). Other venerid species show similar times to attain the pediveliger stage, although larvae are smaller than *C. cortezi*, e.g., *M. meretrix*, and *A. brasiliana* (Narasimham *et al.* 1988; Oliveira *et al.* 2016). In other cases, pediveliger sizes are similar to those of *C. cortezi*, but their development times differ, as in *M. venerijbrmis*, *A. trapezia*, and *R. largillierti* (see Table I) (Nell *et al*., 1994; Kent *et al*., 1999; Hur *et al*., 2005). There are some venerid species in which a functional foot appears much later (after 13 to 20 days PF) and in larvae that are smaller than *C. cortezi*, such as those of *C. stutchburyi*, *R. philippinarum*, and *C. sinensis* (Stephenson & Chanley 1979; Hur *et al.* 2005).

Growth of *C. cortezi* larvae, as described by the relationship between shell L and H is fitted by linear regressions in Figure 3. The predictive equations shown differ in the calculated daily growth rate from those reported for *K. rhytiphora*

(Nell *et al*. 1994), *C. cancellata* (LaBarbera & Chanley 1970), and *M. meretrix* (Narasimham *et al*. 1988). This is expected because the reported larval sizes of these species were in all cases, less than those found for *C. cortezi*. Thus, for venerid species with a comparable rate of larval development to *C. cortezi*, the equations presented in this report and those reviewed from the literature can provide useful baseline data to estimate the daily growth of cultured venerid larvae. These can be used to calculate size-specific feeding rates and thus food delivery, as well as the stocking density in culture systems to achieve improved survival and increase spat production under controlled conditions, which will facilitate commercial mariculture activities.

Once the larvae were ready to metamorphose, they started to lose their velum and settlement occurred. The cumulative survival of *C. cortezi* from hatching to the onset of metamorphosis was 84%, and thus higher than the 60% reported for *A. brasiliana* (Oliveira *et al.* 2016), and especially to the <3% reported for *R. largillierti* (Kent *et al.* 1999). It is known that metamorphosis of *C. cortezi*, *C. cancellata* (D'Asaro, 1967), *C. fluctifraga* (Castillo-Durán *et al.* 2016), *M. meretrix* (Narasimham *et al.* 1988), and *M. opima* (Muthiah *et al.* 2002) occur between day 11 and 13 PF. The reported larval post-settlement sizes were similar, regardless of whether or not a metamorphosis inducer was used. The successful use of neuroactive compounds to induce bivalve settlement such as L-DOPA, a precursor of dopamine, epinephrine and norepinephrine has been well documented (Coon *et al.* 1985; Bonar *et al.* 1990). Induction of metamorphosis with such compounds in venerid clam species has been successful for *R. philippinarum* (Urrutia *et al.* 2004), and speculatively in *C. cortezi*, according to the low mortality reported in the present study; in all cases low mortalities rates (<15%) were reported following metamorphic induction. Cumulative survival through metamorphosis in *C. cortezi* was 44% 2 days after epinephrine induction, a higher value than the minimum of 40% suggested by Utting and Spencer (1991). Higher values have been reported in other studies without settlement inducers, namely 95% for postlarvae of *C. fluctifraga* (Castillo-Durán *et al.* 2016), and 68% in *T. dorsatus* (Nell *et al.* 1995).

Mortality of *C.cortezi* larvae was significant low as opposed to the high mortality observed after epinephrine induction. Survival of *C. cortezi* from metamorphosis until postlarvae attained 2 mm in size was 37%, while mean daily shell growth rate increased to 30.69 μm in L, and 34.09 μm in H. Communication with the producers indicated that the spat grew well, and exhibited a high survival rate of 80% in 2 mm-mesh, bottom culture plastic net bags. The growth results are similar to the 30 μm shell L day^{-1} reported for postlarvae of *C. fluctifraga* (Castillo-Durán *et al.* 2016), although the latter grew to a much larger size (reaching 3 mm at day 60). This may be attributable to their deployment in marine off-bottom systems at a smaller size than that when clams were transferred to the coast of Gulf of California in the present study. A similar mean L of 2 mm was only reached by *M. meretrix* after 75 days of culture in the hatchery (Narasimham *et al.* 1988). Other venerid clam studies indicated that juveniles of *M. opima* were larger than *C. cortezi* after 45 days PF (Muthiah *et al.* 2002). It is very likely that culture conditions (laboratory vs. off-bottom cultivation at sea), microalgal diet, feeding conditions, rearing densities, in addition to species-specific characteristics, are all responsible for the reported growth differences among postlarval venerids. Results showed that if it is desired to improve the laboratory productivity yield of *C. cortezi* clams, it is necessary to increase the surviving percentage during the metamorphosis process, and keep the adequate temperature and salinity according to the habitat conditions of the species. As mentioned for other authors, one option is not to use chemical inductors and use shell powder as have been proved in Venerid clams (Castillo-Durán *et al*., 2016; Nell *et al*., 1995), and in diverse molluscs (Silveira *et al.* 2011; Stott *et al*. 2004). The use of probiotics could be useful too as demonstrated for other bivalves (Kapareiko *et al.* 2011).

This study provides basic biological information about this overfished clam species, which can be readily applied to commercial splat production. It is well established that spat production of native species affected by overfishing, such as *Chione cortezi* in the Gulf of California, provides a means of preventing a decline in ecosystem biodiversity (de Silva, 2012), discouraging the introduction of invasive species and their associated diseases (Diana, 2009), and promotes sustainable mariculture (Diana *et al.* 2013). The present study contributes to our knowledge of the development cycle of *C. cortezi* under laboratory conditions, and demonstrates that it is possible to achieve a level of spat production useful for aquaculture activities in the BC region, and thus allow local producers an

option to maintain productive and sustainable fisheries.

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