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Research Article

Gonadal maturity of *Crassostrea corteziensis* cultivated in the Gulf of California

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ABSTRACT. The gonadal maturation of the pleasure oyster *Crassostrea corteziensis* during four cultivation cycles (November 2004-June 2005, N4J5; August 2014-February 2016, A14F16; September 2015-December 2016, S15D16; September 2016-August 2017, S16A17) was studied in the southeastern Gulf of California, Mexico. Although *C. corteziensis* exhibited the typical reproductive phases (undifferentiated or resting, initial gametogenesis, advanced gametogenesis, maturity, spawning and post-spawning), the intensity of reproduction and the timing of the onset of the different phases of gonadal development varied in each cultivation cycle. Reproductive peaks were observed in different months (N4J5 in May, A14F16 in July, S15D16 in May and S16A17 in June) during spring and summer. Spawning correlated with temperature in the N4J5 and A14F16 cultivation cycles but it showed no correlation with chlorophyll-a concentration in any of the cultivation cycles. *C. corteziensis* presented an opportunistic continuous reproductive strategy with spawning occurring during several months of the year. The modification of the species' reproduction pattern could contribute to the natural settlement of larvae, increasing the natural production of this native oyster resource in the southeast of the Gulf of California, where it has been fished intensively.

Keywords: *Crassostrea corteziensis*; Cortez oyster; reproduction; gametogenesis; spawning; farming; Sinaloa

INTRODUCTION

Studies on reproduction in wild populations of commercially important bivalve mollusks offer valuable information for their management, conservation, and exploitation (Goslin, 2015). When bivalves are cultivated, indicators such as gonad formation and growth help in determining the appropriate time for harvesting (Aníbal *et al.*, 2011). Gonadal development phases are frequently observed throughout the cultivation cycle in bivalve species (Arellano-Martínez *et al.*, 2011; Gomes *et al.*, 2014; Ángel-Dapa *et al.*, 2015). Nevertheless, bivalve reproduction is strongly affected by exogenous factors such as environmental variables that are affected by global warming (Lawrence & Soame, 2004). High temperature (Beukema *et al.*, 2009), low salinity (Peteiro *et al.*, 2018), and high levels of toxic compounds (Guzmán-García *et al.*, 2009) disrupt several metabolic pathways involved in bivalve reproduction. Different reproductive responses can be observed not only between species and localities but also between individuals of the same species at the same site (Hernández-Otero *et al.*, 2014).

The pleasure or Cortez oyster (*Crassostrea corteziensis*) is a bivalve species cultivated semi-intensively in northwest Mexico (Chávez-Villalba *et al.*, 2005). Endemic to this region, *C. corteziensis* is highly valued for consumption and represents an important resource for the oyster industry. Its reproduc-

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tive cycle has been reported for natural (Rodríguez-Jaramillo *et al.*, 2008; Mena-Alcántar *et al.*, 2017) and cultivated populations (Chávez-Villalba *et al.*, 2008; Mazón-Suástegui *et al.*, 2011) from different sites along the northwestern coast of the Mexican Pacific, including the Gulf of California. *C. corteziensis* has an opportunistic reproductive strategy; they focus on nutrient-rich sites where they acquire energy through nutrient consumption, particularly lipids and carbohydrates (Racotta *et al.*, 2008). This strategy allows the pleasure oyster to be reproductively active almost year-round (Hurtado *et al.*, 2012); that is, as a sub-tropical oyster, its annual reproductive pattern is characterized by an extended reproductive period and several partial spawns (Rodríguez-Jaramillo *et al.*, 2017) that lead to variations in reproductive responses.

To our understanding, there is only one study on the gonadal development of a natural population of the pleasure oyster in Sinaloa (Ceuta Lagoon System), Mexico, carried out by Rodríguez-Jaramillo *et al.* (2008). Since 2004 researchers at the Laboratorio de Malacología at the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR)-Unidad Sinaloa, a branch of the Instituto Politécnico Nacional, have been working on obtaining data on gonadal maturity of *C. corteziensis* cultivated in the San Ignacio-Navachiste-Macapule Lagoon System (Sinaloa, Mexico). The present study aimed to analyze the relationship between environmental parameters with gonadal development of the pleasure oyster of four cultivation cycles (from 2004 to 2017) at a farm located in the Macapule Lagoon, in Sinaloa, Mexico and contrasted with previous studies (see references) carried out in other locations in northwestern Mexico. Besides, factors such as initial size, seed origin, and seeding month are discussed.

MATERIALS AND METHODS

Study site and oysters

The cultivation cycles (Nov 2004-Jun 2005, Aug 2014-Feb 2016, Sep 2015-Dec 2016, and Sep 2016-Aug 2017, coded as N4J5, A14F16, S15D16, and S16A17, respectively) were carried out at an oyster farm located in the La Piedra estuary (25°21'-25°24'N, 108°30'-108°45'W), part of the mangrove system in the Macapule Lagoon (Sinaloa, Mexico). The suspended long-line culture system was used in all these studies (Rodríguez-Quiroz *et al.*, 2016). Oyster seeds were produced under laboratory conditions: N4J5 at Centro de Investigaciones Biológicas del Noroeste (CIBNOR), A14F16 and S16A17 at Centro Reproductor de Especies Marinas del Estado de Sonora (CREMES), and S15D16 at Instituto Sinaloense de Acuacultura y Pesca (ISAPESCA) and planted after summer (as routinely done in the region, Rodríguez-Quiroz *et al.*, 2016) and according to the capacity and production availability of each laboratory. All the oyster seed groups (3-4 mm in height shell) were transported to the cultivation site, acclimated following Gallo-García *et al.* (2001), deposited in plastic mesh sacks (2 mm mesh opening), and finally, the sacks were placed onto Nestier™ type culture baskets. The initial density (500 oysters per tray) was thinned to 80 oysters per tray after an initial period (45 days) of field nursery. Oyster seeds reached a juvenile minimum size of 9-10 mm in width, so they could be placed directly in the Nestier™ type culture baskets, which have a hole opening (5 mm) for water exchange. Cleaning and maintenance of trays, ropes, and sacks occurred every 15 days. Table 1 shows the initial conditions of the cultivation cycles.

Environmental parameters

Water temperature, dissolved oxygen (YSY55/12FT Oxymeter, Ohio, USA), salinity (ATAGO, S/Mill refractometer), and pH (Hanna, HI 8314 pHmeter, USA) were recorded monthly during each cultivation cycle. At the same time, depth and transparency were sampled with a Secchi disk. A PVC tube (5 cm diameter and 2 L capacity) fitted with an end plunger seal was used to sample the water column to determine the total suspended solids (TSS), particulate organic matter (POM), and chlorophyll-*a* concentration (Chl-*a*). The gravimetric technique (APHA, 1995) was used to determine the TSS and POM, while the spectrophotometric method (Strickland & Parsons, 1972) was performed to determine the Chl-*a*.

Oyster measurements, survival, and condition index

A total of 50 oysters were taken from the culture system to record the height, thickness, width, and total weight measurements each month. Survival, expressed as a percentage of the initial oyster density, was calculated by separating and counting the empty shells at each sampling. The dry weight of the shells and tissues of 30 oysters (24 h at 100°C) was used to calculate the condition index (CI) defined by Chávez-Villalba *et al.* (2008): $CI = P1 \times 1000 / P2$, where P1 = soft tissue dry weight (g), and P2 = shell dry weight (g).

Histological analysis

The visceral mass (gonad included) of each oyster was dissected, fixed in Davidson's solution for 24 h, dehydrated through a sequence of alcohol, cleared with Hemo-De®, and embedded in Paraplast-Xtra® following Humason (1979). Sections 4 µm thick were cut using a rotary microtome, placed on a slide, stained with Harris haematoxylin-eosin, and examined under a

Table 1. Initial characteristics of the rearing cycles of *Crassostrea corteziensis* cultivated at La Piedra Estuary, Sinaloa, Mexico. CIBNOR: Centro de Investigaciones Biológicas del Noroeste; CREMES: Centro Reproductor de Especies Marinas del Estado de Sonora, ISAPESCA: Instituto Sinaloense de Acuacultura y Pesca. *Different superscripts in the initial shell length denote differences significantly ($P \leq 0.05$). N4J5: Nov 2004-Jun 2005, A14F16: Aug 2014-Feb 2016, S15D16: Sep 2015-Dec, S16A17: Sep 2016-Aug 2017.

Source of seed	Amount of seed stock	Initial shell length (mm)	Cultivation time (months)	Month of sowing
N4J5 CIBNOR	5000	$4.13 \pm 0.67^{c*}$	7	November
A14F16 CREMES	5000	3.87 ± 0.48^{b}	19	August
S15D16 ISAPESCA	4000	3.62 ± 0.58^{a}	17	September
S16A17 CREMES	5000	3.81 ± 0.39^{b}	12	September

light microscope. The gonad preparations were assigned a development stage using categories previously reported for *Crassostrea corteziensis* (Cuevas-Guevara & Martínez-Guerrero, 1979; Baqueiro *et al.*, 1992; Chávez-Villalba *et al.*, 2008) as follows for female/male, respectively: previtellogenesis/early gametogenesis, vitellogenesis/late gametogenesis, mature, spawning, post-spawning, and repose or undifferentiated. Monthly variations in the sex ratio were determined by examining the slides under an optical microscope (40x).

Statistical analyses

All data were analyzed separately for each cultivation cycle, and only the initial oyster size was compared between the four culture cycles. Normality and homoscedasticity were confirmed using the Lilliefors' and Bartlett's tests (Sokal & Rohlf, 1995). ANOVA was used to assess the environmental parameters. Kruskal-Wallis and *post-hoc* Mann-Whitney-Wilcoxon tests were used to detect differences in oyster biometrics, survival, and CI. Percentage data were first subjected to arcsine transformation before the statistical analyses. The correlations between water temperature, Chl-*a*, and spawning were assessed for each rearing cycle. Sex ratio differences were tested using Chi-square tests (x^2) with Yates' continuity correction (Bhujel, 2008). The significant level was set up at $P \leq 0.05$, and Statgraphics Centurion for Windows (ver. 16) was used to perform all statistical analyses.

RESULTS

No differences ($P > 0.05$) were found in the environmental parameters among the cultivation cycles in the La Piedra Estuary, Sinaloa, Mexico (Table 2). The mean values for the four cultivation cycles were: temperature 26.5 ± 3.7°C, DO 5.6 ± 0.9 mg L^{-1}, salinity 32.4 ± 1.9, pH 7.6 ± 0.3, depth 1.5 ± 0.3 m, transparency 0.6 ± 0.2 m, TSS 39.6 ± 13.3 mg L^{-1}, POM 10.2 ± 3.3 mg L^{-1}, and Chl-*a* 5.9 ± 3.4 mg m^3.

There were differences (F = 9.44, $P > 0.0001$) in the initial oyster size of the four culture cycles (Table 1), but the final growth and CI of *Crassostrea corteziensis* were not significantly different ($P \leq 0.05$) for each cultivation cycle (Table 3). The highest shell height (80 mm) and the greatest total weight (55.4 g) were recorded for the N4J5 cultivation cycle. The mean CI was 34.2. Survival ranged from 95% obtained for the N4J5 group to 64% from S16A17 and was significantly different for the S16A17 growth cycle ($P = 0.03$).

Six gonadal development stages were distinguished for cultivation cycles N4J5 and A14F16 (repose/ undifferentiated, previtellogenesis/early gametogenesis, vitellogenesis/late vitellogenesis, mature, spawning, and post-spawning) (Table 4). At the same time, five were observed for S15D16 (undifferentiated, gametogenesis, mature, spawning, and post-spawning) and S16A17 (undifferentiated, early gametogenesis, late gametogenesis, mature, and spawning) (Table 5).

The phases of reproduction of *C. corteziensis* cultivated in La Piedra estuary, Sinaloa, Mexico, at different single culture cycles indicate that spawning was recorded from February (S16A17) to October (A14F16) (Fig. 1). The frequencies of the gonadal development phases for the four farming cycles are shown Fig. 2. All N4J5 individuals were undifferentiated during the first three months. Late gametogenesis was observed from the fourth month (February 2005) onward. Maturation, spawning and post-spawning stages coincided during the last two months. By May and June, approximately 20% of the oysters were in spawning condition (Fig. 2a). Oysters were harvested in July 2005. Spawning and temperature were correlated (r = 0.81, $P = 0.01$). In this cultivation cycle, 4% were hermaphroditic oysters.

The gametogenic development of *C. corteziensis* in the A14F16 cycle started in February 2015 (Fig. 2b)

Table 2. Mean ± standard deviation, minimum, and maximum limits of the environmental parameters at La Piedra Estuary, Sinaloa, Mexico, for the four single cultivation cycles. N4J5: November 2004-June 2005, A14F16: August 2014-February 2016, S15D16: September 2015-December 2016, S16A17: September 2016-August 2017. ND: undetermined. Minimum and maximum limits are indicated in parentheses.

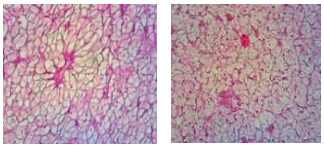
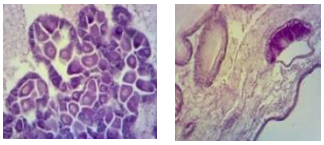
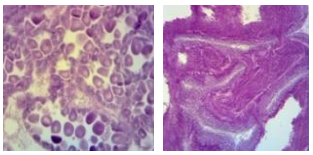
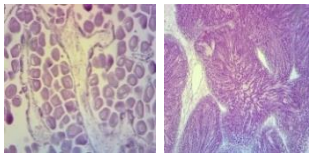
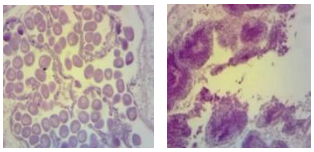
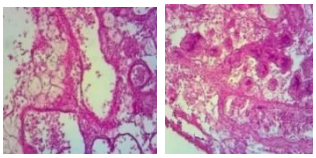
	N4J5	A14F16	S15D16	S16A17
Temperature (°C)	25.5 ± 4.7 (19.0-32.0)	27.2 ± 0.4 (18.8-33.0)	26.0 ± 4.7 (19.1-32.2)	27.5 ± 5.1 (20.0-33.3)
Dissolved oxygen (mg L^{-1})	6.3 ± 1.6 (5.1-7.8)	5.2 ± 0.5 (3.8-6.4)	5.3 ± 0.8 (4.1-6.4)	5.8 ± 1.0 (5.1-7.5)
Salinity	32.1 ± 2.2 (28.0-34.8)	31.3 ± 0.7 (24.5-38.5)	32.8 ± 2.7 (28.0-38.0)	33.6 ± 2.2 (30.0-37.0)
pH	8.3 ± 0.2 (8.1-8.6)	7.7 ± 0.2 (7.1-8.6)	7.4 ± 0.2 (7.1-7.9)	7.1 ± 0.9 (4.8-8.0)
Depth (m)	1.8 ± 0.6 (0.5-0.8)	1.4 ± 0.0 (0.7-2.2)	1.5 ± 0.4 (1.0-2.2)	1.5 ± 0.4 (0.9-2.0)
Transparency (m)	0.5 ± 0.4 (0.2-1.5)	0.5 ± 0.0 (0.2-0.9)	0.6 ± 0.2 (0.2-1.2)	0.7 ± 0.3 (0.3-1.3)
Total suspended solids (mg L^{-1})	ND	43.0 ± 18.3 (23.7-83.2)	36.7 ± 9.6 (25.0-52.2)	39.3 ± 12.1 (22.7-61.6)
Particulate organic matter (mg L^{-1})	ND	10.7 ± 5.0 (2.8-20.0)	9.1 ± 2.5 (6.1-13.3)	10.8 ± 2.4 (6.8-13.8)
Chlorophyll-*a* (mg m^{-3})	ND	7.3 ± 3.3 (3.4-14.2)	4.5 ± 3.3 (1.1-13.0)	6.0 ± 3.7 (2.0-13.0)

Table 3. Mean ± standard deviation of height, thickness, and width of the shell, total body weight, survival, and condition index of *Crassostrea corteziensis* farmed at La Piedra Estuary, Sinaloa, Mexico, for the four single cultivation cycles. N4J5: November 2004-June 2005, A14F16: August 2014-February 2016, S15D16: September 2015-December 2016, S16A17: September 2016-August 2017. ND: undetermined. [1]Month of cultivation in reaching the maximum size. [2]Minimum and maximum limits. [*]Different superscripts in the same raw denote significant differences ($P \leq 0.05$).

	N4J5	A14F16	S15D16	S16A17
Shell height (mm)	70-80 (month 8^{th})[1] ($P < 0.001$)	73.3 ± 9.1 (month 17^{th}) ($P < 0.001$)	71.9 ± 7.6 (month 11^{th}) ($P < 0.001$)	61.5 ± 11.2 (month 11^{th}) ($P < 0.001$)
Shell thickness (mm)	ND	48.8 ± 4.2 ($P < 0.001$)	50.1 ± 4.7 ($P < 0.001$)	46.0 ± 7.5 ($P < 0.001$)
Shell width (mm)	ND	25.6 ± 3.3 ($P < 0.001$)	24.4 ± 4.1 ($P < 0.001$)	21.4 ± 4.5 ($P < 0.001$)
Total weight (g)	55.4 ± 1.1 ($P < 0.001$)	43.6 ± 11.0 ($P < 0.001$)	52.6 ± 10.0 ($P < 0.001$)	50.6 ± 12.4 ($P < 0.001$)
Survival (%)	$95.3 \pm 3.1^{b*}$	ND	94.2 ± 1.8^{b}	64.6 ± 8.5^{a}
CI	ND	32.6 ± 14.8 (21.5-48.6)[2] ($P < 0.008$)	32.2 ± 8.0 (20.4-48.7) ($P < 0.009$)	37.9 ± 14.5 (13.9-69.5) ($P < 0.01$)

with larger specimens (seven months-old oysters, ~70 mm shell height) that were sampled during 13 months of cultivation. Undifferentiated individuals were observed in autumn-winter 2016, indicating a resting phase. *C. corteziensis* in vitellogenesis phases (either previtellogenic or vitellogenic) were found most of the year; meanwhile, mature oysters were detected from May to September 2015. Spawning started in May and

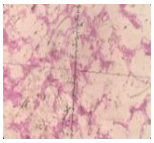
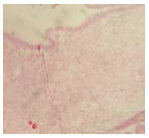
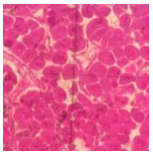
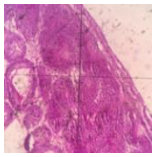
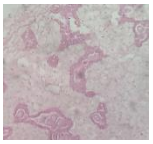
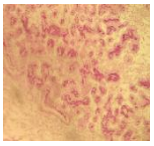
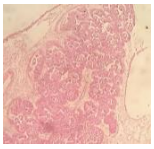
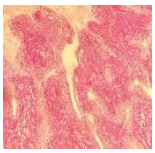
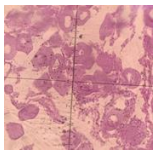
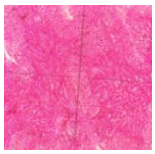
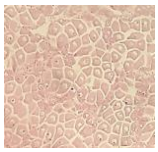
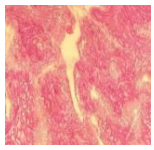
Table 4. Description of gonad developmental stages of *Crassostrea corteziensis* for the N4J5: November 2004-June 2005, and A14F16 cultivation cycles (Hemo-De® and Paraplast-Xtra®, microscopic observation 40 X).

Gonadal stage	Description	
Repose/Undifferentiated	Females (left photo): empty follicles with residual oocytes that can be reabsorbed or remain until next maturation can be observed; abundant connective tissue and phagocytic activity are found. Males (right photo): abundant connective tissue with residual sex cells near the tubules can be found. Phagocytic activity. Empty gonoducts.	
Previtellogenesis Early gametogenesis	Females (left photo): vesicular gametogenic activity is observed in the connective tissue in the form of islets, as well as some immature oocytes attached to the wall of the follicle and few mature oocytes in the gonoduct. Males (right photo): spermatogonias and spermatocytes were differentiated in the periphery and adhered to the follicles. The latter is seen near the lumen in the acinus. Vesicular connective tissue remains abundant.	
Vitellogenesis Late gametogenesis	Females (left photo): there are abundant sex cells arranged in the lumen of large and anastomosed follicles. The follicular walls are thin. Although it is still possible to differentiate oogonia, large pear-shaped oocytes predominate in the acini. Males (right photo): the connective tissue is reduced. The spermatocytes thicken the lumen of the follicle, and the spermatozoa are arranged near the gonoduct.	
Mature	Females (left photo): the oocytes are large, round or polygonal, and have been detached from the follicular wall. They contain abundant and granular cytoplasm. The interfollicular connective tissue is reduced by the growth of follicles. Males (right photo): the follicles are large and distended. Large masses of spermatozoids are distinguished with their tails oriented towards the lumen. A small number of spermatogonia and spermatocytes are observed; the seminiferous tubules are grown and without space between them.	
Spawning	Females (left photo): the follicles are ruptured, and postvitelogenic oocytes are released, leaving spaces in the gonad. Partial spawning can be observed. Males (right photo): the broken follicles are observed, and the spermatozoids are released into the gonoduct. The gonad shows empty spaces.	
Post-spawning	Females (left photo): the expulsion of the oocytes ceases. Large empty spaces in the gonad with remains of oocytes are appreciated. The follicles are broken with phagocytic activity on their walls. Males (right photo): the expulsion of the sperm ceases. Large empty spaces in the gonad with remains of sperm are appreciated. The follicles are broken with phagocytic activity on their walls.	

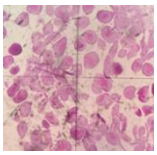
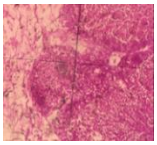
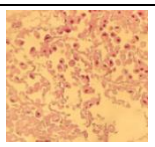
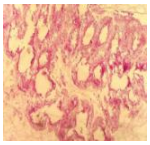
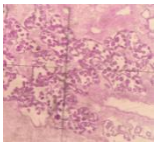
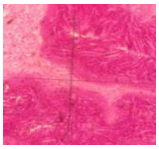
lasted six months (October) with a peak in July. Spawning was correlated with water temperature (r = 0.83, $P < 0.001$) but not with Chl-*a* (r = 0.11, $P = 0.70$). Chl-*a* and water temperature were not correlated (r = 0.31, $P = 0.20$). The sex proportion (F:M = 0.75:1) was significantly different from the expected ratio (x^2 = 133.45, df = 9, $P < 0.001$). In this cultivation cycle, no hermaphrodite specimens were identified.

For the S15D16 farming cycle (Fig. 2c), oysters were in gametogenesis from the second sampling month (September 2015) onward, and this gonadal stage was continuously present until April 2016. Then, gonadal maturation was observed in March and April. Spawned specimens were detected from April to November, but a reproductive peak occurred in April-May. Gonadal development from June to December was characterized by at least 20% post-spawned oysters. Except for April 2015, undifferentiated individuals were observed in all sampling months. In this case, spawning was not correlated with water temperature nor Chl-*a* (r = 0.42, $P = 0.09$ and r = 0.06, $P = 0.82$, respectively). There was no correlation between Chl-*a* and water temperature (r = 0.08, $P = 0.76$). In this farming, the observed sex ratio (F:M = 1:1.42) was significantly different from the expected proportion (x^2 = 123.32, df = 15, $P < 0.001$). The gonads of 2.5% of the oysters analyzed displayed both oocyte and sperm cells.

Table 5. Description of gonad developmental stages of *Crassostrea corteziensis* for the S15D16: September 2015-December 2016, and S16A17: September 2016-August 2017 cultivation cycles (Hemo-De® and Paraplast-Xtra®, microscopic observation 10x, and 40x).

S15D16			S16A17		
Gonadal stage	Description		Gonadal stage	Description	
Undifferentiated	Empty follicles with residual sexual cells are observed; abundant connective tissue and phagocytic activity are found.		Undifferentiated	Since follicles are in formation, sex cells are not differentiated yet; abundant connective tissue is observed. There is not gonadal development.	
Gametogenesis	Females (above): intense vesicular gametogenic activity (islets) is observed; the connective tissue is reduced; small oocytes are attached to the wall of the follicle, and bigger pear-shape oocytes are arranged near the gonoduct. The follicular walls are thin. Males (below): spermatogonias and primary spermatocytes are abundant and differentiated in the periphery of the follicles. Spermatids and matured spermatocytes are seen near the lumen in the follicle. The connective tissue is reduced.	 	Early gametogenesis	Females (above): connective tissue is getting thinner due to the presence of islets; oocytes are attached to the follicular wall; matured oocytes are few but bigger to the primary oocytes and oriented to the gonoduct. Males (below): sex cells of different sizes (spermatogonia and spermatocytes) are located near to the lumen of the acinus or adhered to the follicles. Vesicular connective tissue remains abundant.	 
			Late gametogenesis	Females (above): follicles increase in size due to the abundant sex cells; large pear-shaped oocytes predominate in the acini. Males (below): the vesicular activity (islets) increase, reducing the connective tissue. Also, the thick of the lumen of the islet is reduced. Spermatozoa are arranged near the gonoduct.	 
Mature	Females (above): oocytes are rounded or polygonal, and have been detached from the follicular wall. They contain granular cytoplasm. The interfollicular connective tissue is reduced by the growth of follicles. Males (below): the gonadic tissue has reached its maximum development; follicles are large and distended. Interfollicle walls are not distinguished.	 	Mature	Females (above): the connective tissue has been reduced by the presence of numerous and big follicles with large, round or polygonal oocytes detached from the follicular wall. Oocytes contain granular cytoplasm. Males (below): the follicles are large and anastomosed. Spermatids and spermatozoid cells are located towards the lumen of the follicles.	 

continuation

S15D16			S16A17		
Gonadal stage	Description		Gonadal stage	Description	
Spawning	Females (above): empty spaces within the gonad indicate that follicles are ruptured, and oocytes were released. Partial spawning was observed. Males (below): broken connective tissue is distinguished since sex cells were released to the gonoduct.	 	Spawning	Females (above): the number of oocytes was reduced by spawning. The connective tissue and follicles are ruptured. Spaces in the gonad are observed. Males (below): follicles are broken, and spaces are denoted within the gonad. Sex cells are released, but few spermatozoids remain in the follicles.	 
Post-spawning	Females (above): residual tissues and degraded cells can be observed; phagocytes are found in the follicles; the connective tissue increases. Males (below): the connective tissue grows again, and the gonadic tissue is degraded. Residual sex cells are found in the broken follicles where the phagocytic activity is observed on their walls.	 			

The S16A17 cultivation cycle presented at least two gonadal developmental stages in each month (Fig. 2d). The first sampling month (September 2016) showed oysters in undifferentiated, early gametogenesis, late gametogenesis, and mature phases. Few spawned specimens (10%) were registered in October, but the release of gametes occurred from January to August 2017, with more than 50% of spawned oysters observed in May-June. Similar to the S15D16 growing cycle, no correlation was found between spawning and water temperature (r = 0.35, $P = 0.26$), spawning and Chl-*a* (r = 0.47, $P = 0.12$), and Chl-*a* and water temperature (r = 0.51, $P = 0.08$). There were more females than males (F:M = 1.75:1) throughout the samplings ($x^2 = 86.26$, df = 11, $P < 0.001$). The histological analysis of gonads revealed that 2% of oysters from this growing cycle were hermaphrodites.

DISCUSSION

The reproductive strategy in natural and cultivated populations of bivalves is strongly determined by the specific environmental variables at latitudes they inhabit (Aldana-Aranda *et al.*, 2003; Enríquez-Díaz *et al.*, 2009). However, the reproductive tactics of any species can change because its geographic distribution covers a wide range, as is the case of bivalves that live in temperate (Ángel-Dapa *et al.*, 2015) and sub-tropical zones (Rodríguez-Jaramillo *et al.*, 2017), or due to environmental changes in the region caused by climatic phenomena (García-Domínguez *et al.*, 2011).

In this study, the capacity and production availability of oyster seed of each laboratory strongly determined the seeding month, but as routinely in the region to avoid exposure of juveniles at high temperature (Rodríguez-Quiroz *et al.*, 2016), seeding in the four cultivation cycles was done after summer (August to November). Although the oysters in this study exhibited the typical reproductive phases, there were significant differences in the reproduction intensity and the timing of the onset of gonadal development, which is in agreement with results reported by Chávez-Villalba *et al.* (2003) and Enríquez-Díaz *et al.* (2009) growing *Crassostrea gigas* in several marine areas of France. In the present study, the spawning of *C. corteziensis* cultivated in the southeastern Gulf of California began between January (S16A17) and May (N4J5). Chávez-Villalba *et al.* (2008) and Mazón-Suástegui *et al.* (2011) observed the first spawning in April and August, respectively, in cultivations located at latitudes farther north in the Gulf of California. Barber & Blake (2006) mention that, in particular, water temperature and nutrients are the variables exerting the strongest influence on mollusk reproduction. The range of water temperature reported by Chávez-Villalba *et al.* (2008) of 15-33°C, is wider

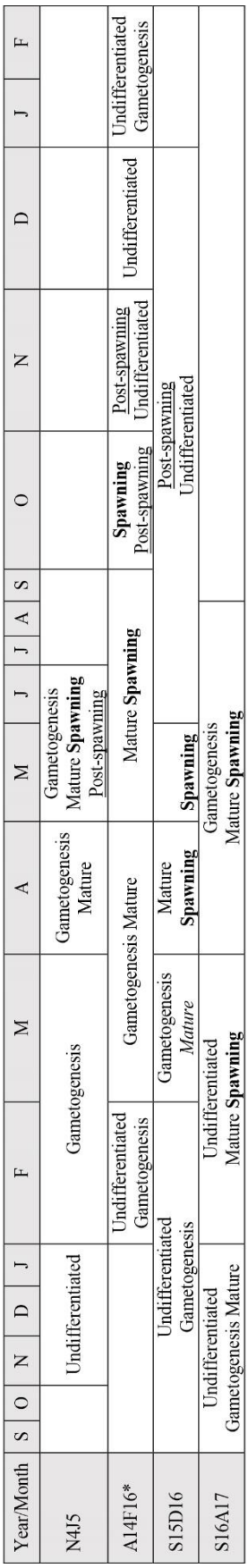


Figure 1. Phases of reproduction of *Crassostrea corteziensis* cultivated in La Piedra estuary, Sinaloa, Mexico, at different single culture cycles. N4J5: Nov 2004-Jun 2005, A14F16: Aug 2014-Feb 2016, S15D16: Sep 2015-Dec, S16A17: Sep 2016-Aug 2017. *Evaluation of gonadal phases in the cultivation cycle A14F16 started on February 2015.

than that recorded by Mazón-Suástegui *et al.* (2011) (22.4-32.2°C) as well as for the cultivation cycles in our study (18.8-33.3°C). Duprat-Bertazzi & García-Domínguez (2005), Enríquez-Díaz *et al.* (2009) and Aldana-Aranda *et al.* (2014) note that spawning in different species of oysters (the rock oyster *Hyotissa hyotis*, the Japanese oyster *C. gigas*, and the eastern oyster *C. virginica*, respectively) are associated with high water temperatures, in agreement with conclusions presented by Mazón-Suástegui *et al.* (2011) but differs from the results reported by Chávez-Villalba *et al.* (2008) and the cultivation cycles reported here. In our study, spawning was registered for the first time during the months when the water temperature was increasing, and reproductive peaks were observed during different summer months (A14F16 in July, S15D16 in May and S16A17 in June) (Table 6).

The temperature data obtained were compared with the National Oceanic and Atmospheric Administration (NOAA) database to assess the presence of positive (El Niño Southern Oscillation, ENSO) or negative (La Niña weather phenomena) anomalies during the productive cycles that might influence *C. corteziensis* gonadal development. According to the values of the Oceanic Niño Index (ONI), a weak ENSO was registered from November 2014 to May 2016 followed by two normal months (June and July); then, a low-intensity La Niña of the low episode occurred from August to December 2016 (CPC, 2018). The reproductive process of the pleasure oyster between 2014 and 2017 presented a distinct seasonal pattern with maximum spawning peaks in May and June. However, the spawning intensity was lower when the La Niña phenomenon occurred; that is, during the S16A17 culture cycle, indicating that low water temperatures at the beginning of spawning could delay the reproductive activity of the pleasure oyster, as also concluded by Magaña-Carrasco *et al.* (2018) for *C. virginica*.

During the three most extended cultivation cycles, the Chl-*a* concentration ranged from 1.1 to 14.2 mg m^{-3}. However, it did not show a typical seasonal pattern. This range was lower than that reported by Chávez-Villalba *et al.* (2008) (~0-28.5 mg m^{-3}) at latitudes north of our cultivation site, but similar to that recorded by Rodríguez-Jaramillo *et al.* (2008) (~1-15 mg m^{-3}) in the lagoon system in Ceuta, Sinaloa. In the S16A17 cycle, the Chl-*a* peak was observed before spawning. Meanwhile, the highest Chl-*a* was obtained during spawning for A14F16, and four months after spawning for S15D16. Besides, the higher Chl-*a* values did not coincide with the summer of each year, in contrast to the results reported by Chávez-Villalba *et al.* (2008). It is characteristic of some species of mollusks that before

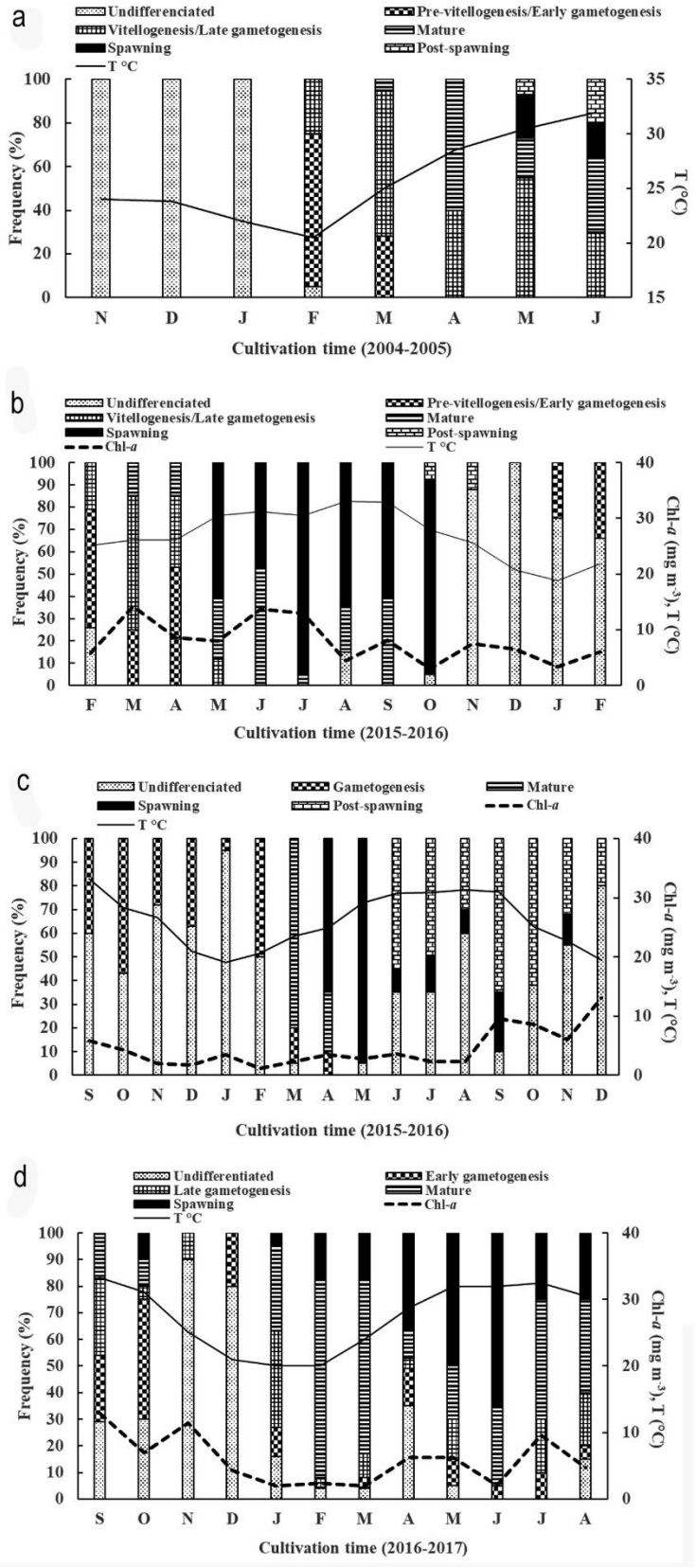


Figure 2. Frequency of the gonadic developmental stages of *Crassostrea corteziensis* cultivated in La Piedra Estuary, Sinaloa, Mexico, at different single culture cycles. a) N4J5, b) A14F16, c) S15D16, d) S16A17. N4J5 = Nov 2004-Jun 2005, A14F16 = Aug 2014-Feb 2016, S15D16 = Sep 2015-Dec, S16A17 = Sep 2016-Aug 2017.

Table 6. Comparison of different reports on the reproductive cycle of *Crassostrea corteziensis* in north western Mexico.

Site	Sampling time	Origin	Developmental phases	Release of gametes	Reproductive peak	Sex ratio F:M	Reference
Ceuta, Sinaloa	Ap 2005-Ap 2006	Wild	Undifferentiated (Stage 0); previtellogenesis/early gametogénesis (Stage I); vitellogenesis/late gametogenesis (Stage II); postvitellogenesis/mature (Stage III); spawned (Stage IV)	April to December	May to October	1:0.35	Rodríguez-Jaramillo *et al.* (2008)
Camichín, Nayarit	Sep 2013-Aug 2014	Wild	Undifferentiated; development; ripe; spawning; post-spawning (George-Zamora *et al.*, 2003)	Sept-Nov and March-Aug	May, August, and October	1:0.32	Mena-Alcántar *et al.* (2017)
Guaymas, Sonora	Nov 2002-Nov 2003	Cultivated	ND	ND	June and September, determined with the condition index	ND	Chávez-Villalba *et al.* (2005)
Las Guásimas, Sonora	Jul 2003-Sep 2005	Cultivated	Early gametogenesis; growing; mature; degenerating (Lango-Reynoso *et al.*, 2000 for females; Chávez-Villalba *et al.*, 2002 for males)	March 2004 and April 2005 to November 2004 and 2005	October 2003/April 2004/June 2005	1:0.68	Chávez-Villalba *et al.* (2008)
Bahía Agiabampo, Sonora	Jan-Oct 2005	Cultivated	Undifferentiated; previtellogenesis; vitellogenesis; postvitellogenesis; spawned (Rodríguez-Jaramillo *et al.*, 2008)	August to October	Spring/Summer	1:1.17	Mazón-Suástegui *et al.* (2011)
La Piedra, Sinaloa	Nov 2004-Jun 2005	Cultivated	Early gametogenesis; late gametogenesis; mature, spawning; post-spawning; undifferentiated/repose (Cuevas-Guevara & Martínez-Guerrero, 1979)	February to June	May to June	1:2.14	This study
La Piedra, Sinaloa	Feb 2015-Feb 2016	Cultivated	Undifferentiated/repose; early gametogenesis; late gametogenesis; mature; spawning; post-spawning (Chávez-Villalba *et al.*, 2008)	May to October	July to October	1:1.32	This study
La Piedra, Sinaloa	Sep 2015-Dec 2016	Cultivated	Early gametogénesis; late gametogénesis; mature; reproductive/spawning; post-spawning; undifferentiated/repose (Baqueiro *et al.*, 1992; Sevilla, 1993)	April to November	April to May	1:1.42	This study
La Piedra, Sinaloa	Sep 2016-Aug 2017	Cultivated	Undifferentiated/repose; early gametogenesis; late gametogenesis; mature; spawning (Chávez-Villalba *et al.*, 2008)	January to August	April to June	1:1.75	This study

spawning, organisms store food reserve components that are used in gonadal growth and development (Rodríguez-Astudillo *et al.*, 2005; Dridi *et al.*, 2007; Aldana-Aranda *et al.*, 2014), which is a distinguishing characteristic of bivalves with a conservative and seasonal reproductive strategy. In our study, *C. corteziensis* spawning evaluated from 2014 to 2017 was detected between six to nine months per year and no correlated with the Chl-*a*, indicating an opportunistic breeding strategy; this coincides with observations made by Rodríguez-Jaramillo *et al.* (2008) and Mena-Alcántar *et al.* (2017) for the same species.

On the other hand, it is important to consider not only the quantity but also the quality of nutrients. As for filtering organisms, oysters ingest phytoplankton (Hurtado *et al.*, 2012) and seston not related to chlorophyll (particles rich in organic matter) (Chávez-Villalba *et al.*, 2005). The nutritional variety provided by these inputs fulfills all of the pleasure oyster's metabolic requirements (reproduction, growth). Differences in food availability and quality during our farming cycles compared with those reported in other studies could explain the variation in the results during the gametogenic cycle. The other physical, chemical and biological parameters presented adequate ranges for oyster cultivation in the region (Rodríguez-Jaramillo *et al.*, 2008; Chávez-Villalba, 2014).

Oyster seed used in the four cultivation cycles were produced under controlled conditions at each laboratory (CREMES, CIBNOR, ISAPESCA), which imply broodstock management and food production (microalgae), among other standard technological procedures (Ramos *et al.*, 2013). Nevertheless, some specific aspects not available for the farmers (broodstock origin and genetics, microalgae species and concentrations, culture conditions) may have been different among them varying seed quality also. The similar results obtained in the four cultivation cycles suggest that the growth and reproductive performance of *C. corteziensis* were not affected by the seed produced in the different laboratories.

The effect of different initial size grades of oysters on final growth and size is well documented (Foltz & Chatry, 1986; Mason *et al.*, 1998). For instance, Hand *et al.* (1999) concluded that the initial size grade of diploid and triploid rock oyster *Saccostrea commercialis* had a significant effect on final mean whole weight and shell height for both ploidy types, which does not match the present study. Although significant differences were found between the initial sizes of *C. corteziensis* in the four culture cycles, the final size and weight of oysters were similar regardless of the duration of the cultivation time. Kraeuter *et al.* (2007) mention that the growth rate of young *C. virginica* oysters is high but substantially declines once it reaches 50-60 mm shell length. Coinciding with the observations in this study, in which small pleasure oysters grew faster during the first culture months, reproduced and finally, the growth rate declined and stabilized given similar final sizes and weights at the end of each cultivation year in La Pitahaya Estuary. Earlier studies culturing *C. gigas* (Rodríguez-Quiroz *et al.*, 2016; Villanueva-Fonseca *et al.*, 2017) and *C. corteziensis* (Góngora-Gómez *et al.*, 2018) in the same estuary reported a similarly rapid growth rate pattern at the first culture months, suggesting that more than oyster seed size, the estuary environmental conditions strongly affect oyster growth.

The oysters in cultivation cycles N4J5, A14F16, and S15D16 had a shell height of 70-80 mm between the 8th and 17th cultivation months, which is very similar to Chávez-Villalba *et al.* (2005, 2008) report of a shell height of 71.3 mm at 13 months and of approximately 80 mm at 15 months when cultivating *C. corteziensis* in different locations in the Gulf of California. The maximum shell height in S16A17 was attained in the 11th month (61.5 mm). The genetic and seed sources could partially explain the difference in growth (Gutiérrez *et al.*, 2018); however, the oysters used in A14F16 were from the same laboratory, which suggests that environmental parameters exerted a greater influence on *C. corteziensis* growth, as suggested by Hughes *et al.* (2017) for *C. virginica*. Previously, we noted that the low water temperatures registered at the beginning of that cultivation cycle could influence the pleasure oyster's reproductive activity resulting in the lower values obtained for survival and shell height. Chávez-Villalba *et al.* (2005) concluded that low temperatures inhibit *C. corteziensis* development, which is similar to our results.

As in all bivalves, *C. corteziensis* reproduction is defined by the sequence of gonadal development phases; however, several distinct classifications systems are used to identify these phases in this species (Table 6) (Cuevas-Guevara & Martínez-Guerrero, 1979; George-Zamora *et al.*, 2003; Osuna, 2006). Indeed, several classifications separate the description of some gonadal phases by sex (Baqueiro *et al.*, 1992; Sevilla, 1993; Chávez-Villalba *et al.*, 2008). In organisms with an opportunistic reproductive strategy with partial spawning and reproduction peaks throughout the year, the determination of gonadal development stages is difficult under microscopic observation because up to five phases can be differentiated in one sampling month, as occurred with S16A17.

Sexual reversion among bivalves is related to size (Lee *et al.*, 2012) and age (Deslous-Paoli & Héral,

1988). The sex change has been reported for several oyster species (Mann, 1979; Gosling, 2004), including *C. corteziensis* (Chávez-Villalba *et al.*, 2008). The sex ratios observed in our four cultivation cycles showed a higher proportion of males than females, coinciding with results published by Mazón-Suástegui *et al.* (2011), but differ from those of Chávez-Villalba *et al.* (2008), Rodríguez-Jaramillo *et al.* (2008) and Mena-Alcántar *et al.* (2017) for the same oyster species. Baqueiro-Cárdenas (1991) described *C. corteziensis* as a hermaphroditic protandric species, being the first males when they are small and females when they grow larger, which also was observed in our study. The presence of hermaphroditic oysters in three of the four cultivation cycles is a common condition reported for protandric species (Chávez-Villalba *et al.*, 2008) like *C. corteziensis*. Lango-Reynoso *et al.* (2006) mention that the appearance of hermaphrodites coincides with the stage of sexual transition in *C. gigas*, that is, when they are young or small. The proportion of hermaphrodites in our rearing cycles were greater than those reported for other species of oysters such as *C. rhizophorae* (0.8-1.3%) (Lenz & Boehs, 2011) and *C. virginica* (0.83%) (George-Zamora *et al.*, 2003); indeed, the proportion of hermaphroditic *C. corteziensis* in our study exceeded that reported by other researchers for the same species (1.5%) (Chávez-Villalba *et al.*, 2008; Mena-Alcántar *et al.*, 2017). These distinct results may be due to differences in age (Park *et al.*, 2012), species (Kasaynov, 2001), genetics (Gutiérrez *et al.*, 2018), and the specific environmental conditions (Yusa, 2007) at each locality.

Constant monitoring and evaluation of other reproductive indicators such as histochemistry (Rodríguez-Jaramillo *et al.*, 2008) and the diameter and oocyte diameter in the different phases of *C. corteziensis* development (Lango-Reynoso *et al.*, 2000) in culture, are recommended in order to predict their reproductive seasonality accurately; this could improve larval recruitment in a natural environment facilitating better management of the species.

Our study revealed several important aspects of *C. corteziensis* development and reproduction. First, except for the A14F16 cultivation cycle, spawning was not significantly related to water temperature or food availability (Chl-*a*), suggesting that these variables at La Piedra Estuary region are not limited for oyster reproduction. Second, the reproductive peaks did not coincide with the maximum water temperature; instead, as water temperature increased, *C. corteziensis* presented a continuous opportunistic reproductive strategy with spawnings several months a year. Third, when sowing in November at the La Piedra Estuary region, oysters could be harvested between March and April, just before spawning and with the best condition index, palatability, and market price. Fourth, the spawning intensity was lower during the S16A17 culture cycle, indicating that low water temperatures at the beginning of spawning could delay oyster reproductive activity. Finally, under hatchery conditions, oyster broodstock can be induced to mature sexually and to spawn outside their natural reproductive season, so the oyster seed can be produced and sown at different times of the year, which could extend the reproductive period of cultivated *C. corteziensis*. If there are several spawning events per year, the cultivation of the species from hatchery seed could increase the production of larvae and their natural settlement in the wild for more months in a year, thus contributing to the recovery of the oyster resource in a region of Mexico, where it has been heavily exploited.

ACKNOWLEDGMENTS

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