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Short Communication

Reproduction of Cortez oyster, *Crassostrea corteziensis* (Hertlein, 1951) in a growing area in the central Mexican Pacific coast

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ABSTRACT. The state of Nayarit is the main oyster producer in the Mexican Pacific, this derived from artisanal crops of *Crassostrea corteziensis* and collecting of wild seeds. Despite its importance, there are no studies on the reproduction of this species in Boca de Camichín, Nayarit, Mexico, which is the area with the highest production (around 90%). The aim of this study was to evaluate sex ratio, condition index (CI), reproductive cycle, and recruitment size for reproduction and its relationship with environmental factors. Sixty oysters were sampled monthly at La Palicienta Lagoon for one year. The organisms were weighed and measured, then fixed with 10% formalin for histological analyses. Subsequently, the gonads were processed by histology. Sex ratio was different from 1:1, with a predominance of females. The condition index (CI) was higher from March to July. Oyster spawning was most prevalent in May, August, and October. The temperature had a positive relationship with reproduction. Recruitment size at reproduction was 57.1 mm. The results of this study could supply useful information about reproduction of the species at La Palicienta and seed collection in Boca de Camichín, Nayarit.

Keywords: *Crassostrea corteziensis***,** reproduction, seed collection, condition index, temperature influence, Mexican Pacific.

In Mexico, the production of oysters is one of the most important fishing activities (Sevilla, 1993). However, a stagnation in oyster production has been observed at the national level from 2000 (51, 315 ton) to 2013 (42, 524 ton) with a tendency to decrease. Nayarit is the third national producer of oysters in Mexico and is the first producer in Mexico's Pacific Coast, reaching in 2013 a total production of >2, 400 ton, which means 43% of the total production of this littoral (SAGARPA, 2015). This production derives mainly from artisanal crops of Cortez oyster *Crassostrea corteziensis*. In Nayarit State, the aquaculture of *C. corteziensis* consists of the collection of seeds in sea shell substrates, which are attached to nylon monofilament cords or metal wire strings hanging on mangrove wood posts until they reach an adequate size for consumption (60-70 mm) (Chávez-Villalba *et al*., 2005); however, to our knowledge, no studies have been reported in the zone about oyster reproduction. To support wild seed collection for aquaculture purposes and commercial fishing exploitation of Cortez oyster, it is important to make research on their reproductive cycle and the environmental factors that govern its reproduction.

The reproductive cycle and the influence of abiotic variables have been characterized on the reproduction of *C. corteziensis* in many coastal lagoons of Mexico (Cuevas-Guevara & Martínez-Guerrero, 1979; Chávez-Villalba *et al*., 2008; Rodríguez-Jaramillo *et al*., 2008; Mazón-Suástegui *et al*., 2011). For example, in Las Guasimas Lagoon, Sonora State, Mexico, temperature has been reported as the main exogenous factor affecting the reproductive cycle (Chávez-Villalba *et al*., 2008). Mazón-Suástegui *et al*. (2011) reported that maximal reproductive activity of *C. corteziensis* occurs when there is a peak in the content of inorganic particulate matter in Bahía de Agiabampo, Sonora, Mexico. However, in the estuarine system of Boca de Camichín; which is the area that produces over 90% of Cortez oyster in Nayarit, there are not studies on the reproductive biology of this species. The aim of this

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study was to evaluate the reproduction (sexual proportion, size at first maturity, condition index, and gonadic maturity stages) of Cortez oyster and its relationship with environmental variables in La Palicienta Lagoon, which is the seed collection site in the estuarine system of Boca de Camichín, Nayarit, Mexico.

Oysters for this study were sampled at La Palicienta Lagoon, Boca de Camichín, Nayarit, México (21°43' 26'' - 21°45'41''N and 105°29'2'' - 105°30'22''W). Sixty wild oysters with lengths sizes between 20 and 100 mm were sampled monthly from mangrove roots between September 2013 and August 2014. The organisms were washed and scrubbed to eliminate epibionts, then the size (wide, length, height) and total weight of each oyster were registered. The oyster meat was removed from the shell and weighed. Then, 30 specimens from each sample were dried in an oven (65°C/48 h) to determinate dry tissue weight, and the remaining 30 were fixed in 10% formalin and stored for histological analyses. Also, wet and dry shell weights were determined. During each sampling, temperature and salinity were recorded with a potentiometer and a refractometer, respectively. We obtained 2 L^{-1} of water at the time of the sampling for chlorophyll-*a* (Chl-*a*) determinations using standard spectrophotometry (Parsons *et al*., 1984). Seston (organic and inorganic particulate matter) was obtained by calcination and gravimetry (Chávez-Villalba *et al*., 2005). Sex ratio was estimated by dividing the number of females among the numbers of males. A Chi-square (χ^2) test was applied to determine whether the sex ratio differed from 1:1. The tissues and shells of 30 specimens were used to calculate the condition index (CI) described by Walne & Mann (1975). The Condition Index (CI) was calculated as the proportion of dry tissue weight (DTW) with respect to dry shell weight (DSW) by the following formula: $CI = w_1 \times 1000\ w_2^{-1}$, where w_1 is the DTW and w_2 is the DSW. Gonadal developmental stages were estimated by histology (Hematoxylin-Eosin). Were considered five stages (undifferentiated, development, ripe, spawning, and post-spawning) according to George-Zamora *et al*. (2003). The monthly relative-frequency of the stages of gonadal development of males and females were calculated. Recruitment size at reproduction was considered when 50% of the specimens presented gonadal maturity (Mazón-Suástegui *et al*., 2011) and spawning stages, using a logistic model. The relative frequency and frequency-interval accumulated length (5 mm) was calculated. The logistic model was adjusted as follows:

$$M\% = \frac{a}{1 + be^{-cL}}$$

where: M%: percentage of mature specimens, a: intercept, b: slope, c: constant, and L: total length. To determine the relationship of each stage of the reproductive cycle (relative frequency) with each of the measured variables, a Spearman correlation analysis was applied. Statistical analyzes were performed with Statistica 7.1 software (StatSoft).

Environmental parameters recorded during the sampling period of this study are shown in Table 1. The surface water temperature ranged from 25.5°C (December) to 33.0°C (July). Chl-*a* concentration ranged between 0.000 (April and August) to 17.506 mg m^{-3} (February). Salinity was lower in summer (4 g L^{-1} in September) and higher in late winter and early spring (33-35 g L^{-1} from February to April). Finally, particulate organic matter varied from 0.0016 to 0.0317 mg L^{-1} between September 2013 and August 2014 and inorganic matter from 0.0064 to 0.1637 mg L^{-1} for the same months (Table 1).

However, according to the x^2, sex ratio was only different from 1:1 in October (x^2 = 12.0; P = 0.00), March (x^2 = 8.0; P = 0.00), April (x^2 = 3.77; P = 0.05), and July (x^2 = 4.27; P = 0.03). In May, hermaphrodite organisms were found in a percentage of 1.28% with respect to the number of samples obtained. Our results did not show a positive relation between CI and the reproductive process (Table 3). For the sexual proportion was observed that females predominated (Table 2).

A resting period from December to February was observed (Fig. 1). Two peaks of oyster spawning occurred during September-November, and March-August, but mass spawning peaks occurred in May, August and October (Fig. 1).

Temperature plays an important role in the reproductive cycle, followed by Chl-*a*. Spawning stage was predominant in months when the temperature is normally higher (R = 0.90; P = 0.00) (Table 3). The concentration of Chl-*a* showed a negative relationship with gametogenesis (R = –0.76; P = 0.00) during the reproductive period (Table 3). CI was related with salinity (R = 0.60; P = 0.03) (Table 3).

The remaining variables showed no correlation with CI in *C. corteziensis* in La Palicienta Lagoon (Table 3). The interval of length at which mature organisms occurred in Cortez oyster was 28-96 mm. However, recruitment size at reproduction (L_{50}) was 57.1 mm (Fig. 2).

This work recorded that female generally predominate throughout the year. This finding is similar to that reported by several authors in oysters of the genus *Crassostrea* (Rodríguez-Jaramillo *et al*., 2008; Lenz & Boehs, 2011). The age and size of the

Table 1. Environmental factors average values during the sampling period (September 2013 to August 2014), at La Palicienta Lagoon, Boca de Camichín, Nayarit, México. OM: organic matter, IM: inorganic matter.

Month	Environmental factors				
	Temp (°C)	Chl-*a* (mg m^{-3})	Salinity (g L^{-1})	OM (g L^{-1})	IM (g L^{-1})
September	29.3	10.1385	4	0.0317	0.1637
October	30.7	9.5793	25	0.0147	0.0561
November	27.6	4.0473	33	0.0138	0.0377
December	25.5	5.6662	12	0.005	0.0143
January	25.8	13.3714	21	0.0055	0.0178
February	28.2	17.5067	33	0.0086	0.0421
March	27.5	2.2885	34	0.0109	0.0456
April	25.9	0.0000	35	0.0196	0.0573
May	30.9	9.969	34	0.0145	0.0465
June	29.7	4.0003	19	0.0034	0.0117
July	33.0	0.8885	32	0.0031	0.0144
August	31.0	0.0000	14	0.0016	0.0064

Table 2. Females (F), males (M), and sexually undifferentiated (U) Cortez oyster *Crassostrea corteziensis* at La Palicienta Lagoon, Boca de Camichín, Nayarit, México (Sept/2013 to Aug/2014). *Denoted significant difference ($P < 0.05$).

Month	F	M	U	X^2	*P*
September	20	10	0	1.67	0.2
October	24	0	0	12	0.00*
November	16	4	6	3.6	0.06
December	4	2	18	0.33	0.57
January	6	2	20	1	0.32
February	4	4	24	0	1.00
March	16	0	10	8	0.00*
April	20	6	4	3.77	0.05*
May	14	8	0	0.82	0.37
June	20	8	0	2.57	0.11
July	22	6	0	4.57	0.03*
August	18	10	0	1.14	0.29
Total	184	60	82	31.51	0.00*

Table 3. Correlation between environmental variables and reproduction of Cortez oyster *Crassostrea corteziensis* at La Palicienta Lagoon, Boca de Camichín, Nayarit, México (Sept/2013 to Aug/2014). CI: Condition Index. *Denoted significant difference ($P < 0.05$).

Variables	Spearman *R*	*P*
Temperature & Undifferentiated	-0.8173	0.0011*
Temperature & Spawning	0.9065	0.0000*
Chlorophyll-*a* & Development	-0.7614	0.0040*
CI & Undifferentiated	-0.1306	0.6857
CI & Development	0.2136	0.5049
CI & Chlorophyll-*a*	-0.2696	0.3965
CI & Temperature	0.1958	0.5419
CI & Inorganic Matter	-0.0349	0.914
CI & Organic Matter	-0.1188	0.7128
CI & Salinity	0.6024	0.0381*

organisms can be a determining factor for sex predominance, because the younger and smaller individuals are usually males and the older and larger organisms are females (Vélez, 1991). In this sense Lango-Reynoso *et al*. (2006) report that hermaphrodites comprise the intermediate stage of male-to-female transition in *C. gigas*. This can be attributed to the protandrous condition that some species of this genus undergo (Chávez-Villalba *et al.*, 2008). In this work, it was found a 1.3% hermaphroditic specimens in May, which is the month of greatest reproductive activity for *C. corteziensis*, at La Palicienta Lagoon. The proportion of hermaphrodites (1.5%) is similar to that reported for this species in other areas of distribution (Chávez-Villalba *et al*., 2008) but greater than other species of the same genus, such as *C. virginica* (0.83%) (George-Zamora *et al*., 2003), and *C. rhizophorae* (0.8 to 1.3%) (Lenz & Boehs, 2011). These authors attribute hermaphroditism to the age of specimens and to environmental stimuli; however, Paniagua-Chávez & Acosta-Ruíz (1995) attributed this to the fact that in hermaphroditism, complete removal and reabsorption of gametes of the most recent reproductive cycle and new gametogenesis do not occur. The condition index (CI) can evaluate parameters, such as nutritional status, temporally changes in nutritional reserves, and marketable quality (Chávez-Villalba *et al*., 2007) and further found about a relationship between the CI and sexually mature bivalves have been reported (Okumus & Stirling, 1998). However, in this study, no correlation between the CI and sexual maturity was found in

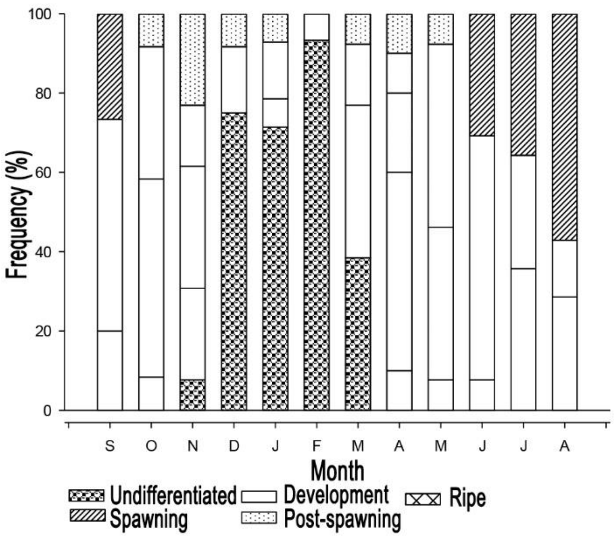

Figure 1. Gonadic developmental stages of Cortez oyster *C. corteziensis* in La Palicienta Lagoon, Boca de Camichín, Nayarit, México (September 2013 to August 2014).

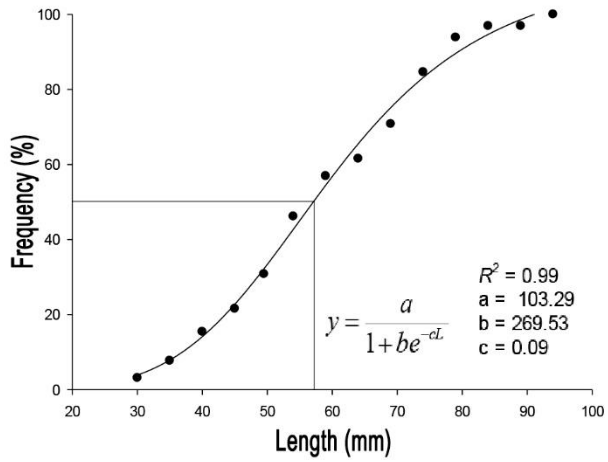

Figure 2. Recruitment size at reproduction of the Cortez oyster *C. corteziensis* at La Palicienta Lagoon, Boca de Camichín, Nayarit, México (September 2013 to August 2014).

C. corteziensis, as reported by Chávez-Villalba *et al*. (2007) and Lango-Reynoso *et al*. (1999) for *C. gigas*. In this study, an increased CI was found in the months of January and February, when the highest values of chlorophyll-*a* were also found, this coinciding with the point made by Thompson & MacDonald (1991), who showed the temporal separation between the reserves accumulation and gamete production in temperate bivalves, as noted by Chávez-Villalba *et al*. (2005) for *C. corteziensis* in Bahía de San Francisco, Sonora. We coincide with the last authors in the sense that the autumn-winter period is the time when some temperate species such as *C. corteziensis* build reserves, which are subsequently employed for gametes development.

Predominant spawning of species occurs in May, August, and October. Development stages is predominant in March, April, and July, and a resting period occurs from December to February. This is similar to that found for this species both in Nayarit and in other localities of the Mexican Pacific (Cuevas-Guevara & Martínez-Guerrero, 1979; Chávez-Villalba *et al*., 2008; Rodríguez-Jaramillo *et al*., 2008; Mazón-Suástegui *et al*., 2011). The occurrence of development stage and spawning during these months is attributable to the increase in water temperature, which is the environmental factor that exerts most control over the physiology of oysters (Chávez-Villalba *et al*., 2005). Temperature has been proved to be the main environmental factor regulating reproduction in marine bivalves (Thompson *et al*., 1996). For *C. corteziensis*, several authors have reported a temperature-related pattern in different parts of the Mexican Pacific; partial spawning occurs during temperature descents and steep increases, while mass spawning takes place at the maximal annual temperatures reported (Rodríguez-Jaramillo *et al*., 2008; Mazón-Suástegui *et al*., 2011). In this study, spawning was observed from March to November, but was more important in May and October, when abrupt temperature increases were recorded. The temperature range within *C. corteziensis* spawned at La Palicienta Lagoon, Boca de Camichín, Nayarit, México was 25.9-33°C. Cuevas-Guevara & Martínez-Guerrero (1979) found that temperature is a determining environmental factor for spawning and that *C. corteziensis* only spawn at temperatures above 25.5°C. In addition to temperature, it has been reported that this is related with other environmental factors. In this work, a negative correlation between gonadal development and Chl-*a* concentration was observed which coincides with the lowest temperature recorded. Similar results are reported by Rodríguez-Jaramillo *et al*. (2008) and Chávez-Villalba *et al*. (2008) for *C. corteziensis* and by Chávez-Villalba *et al*. (2002) for *C. gigas*. Higher levels of Chl-*a* and particulate organic matter corresponded to a period of gonadal rest in winter, during which the oyster increased its nutrient and energy reserves, which can be employed to start the gametogenic cycle. Recruitment size at reproduction has been defined in different ways. Mazón-Suástegui *et al*. (2011) defined it as the time when 50% of the population is in a stage of maturity or when the sum of the stages of maturity and spawning is ≥50%. We define recruitment size in the same manner as these authors. Different sizes at maturity have been established for *C. corteziensis*; Mazón-Suástegui *et al*. (2009) found developing gametes from 10 mm shell height in a hatchery cultivated juvenile population. Chávez-Villalba *et al*. (2008) found mature gonads between 45 and 50 mm in shell height, and Mazón-Suástegui *et al*. (2011) found mature males from 42 mm in shell height and females from a shell height of 54 mm, under culture conditions in the field, and from hatchery seed production. In this study, we found that recruitment size at reproduction for *C. corteziensis* is 57.1 mm in shell height. The first sexual maturity depending on a complex interaction of endogenous and exogenous cues including genetic mechanisms triggered at the onset of gametogenesis (Cruz *et al*., 2000), temperature variations and availability of food related to the geographic range of populations of the same species (Chung *et al*., 2005).

In conclusion, this study shows that food availably was not related with sexual maturity or spawning, while the temperature is the environmental factor that determines the spawning events in Cortez oyster *Crassostrea corteziensis* in the study area. Reproductive activity occurs between 25.9 and 33°C but with greater intensity when abrupt temperature changes occur reaching average temperatures between 30 and 31°C. Therefore, it is recommended to producers in La Palicienta Lagoon to place the seed collectors when the surface temperature reaches 29°C to wait for the spawns and to collect the oyster seed.

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