

ARTICLE

# Reproductive aspects of *Chiton virgulatus* in Bahía de La Paz, Gulf of California, Mexico

Aspectos reproductivos de *Chiton virgulatus* en Bahía de La Paz, Golfo de California, México

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**Resumen.** Se analizó el ciclo reproductivo de *Chiton virgulatus* y su relación con la temperatura superficial del mar y aire (TSM, TA), el fotoperiodo y el ciclo de mareas (CM) en Bahía de La Paz en la costa del Golfo de California. Se tomaron muestras mensuales de septiembre de 2008 a septiembre de 2009. Se registró la longitud, el peso total y peso de la gónada de cada organismo, la gónada fue procesada usando una técnica histológica, se estimó el índice gonádico y la longitud de primera madurez (L50). *C. virgulatus* presenta desoves parciales con un pico reproductivo en verano y otoño (julio-diciembre). La frecuencia de organismos maduros estuvo asociada con los aumentos de TSM (30,6 °C), TA (29,9 °C) y CM (68 cm, pleamar), mientras que el fotoperiodo (días más largos) moduló la frecuencia de organismos en desarrollo. La longitud de primera madurez fue de 29,7 mm. *Chiton virgulatus* es un poliplacóforo al que las altas temperaturas y el nivel de marea alto, influyen en el momento reproductivo, mientras que un aumento en la luz del día favorece el desarrollo gonádico.

**Palabras clave:** Gónada, factores ambientales, Poliplacóforos, talla de primera madurez

**Abstract.** This study analyzed the reproductive cycle of *Chiton virgulatus* and its relationship with air temperature (AT) sea and surface temperature (SST), photoperiod, and tidal cycle (TC) in Bahía de La Paz, in the coast of the Gulf of California. Monthly samples were collected from September 2008 to September 2009. The parameters recorded included total length, total weight, and gonad weight of each organism; the gonad was processed using a histological technique, and the gonadal index and length at first maturity (L50) were estimated. *C. virgulatus* displays partial spawning with a reproductive peak in summer and autumn (July to December). The frequency of ripe organisms was associated with rises in SST (30.6 °C), AT (29.9 °C), and TC (68 cm, high tidal), while photoperiod (longer days) regulated the frequency of developing organisms. The length at first maturity was 29.7 mm. *Chiton virgulatus* is a polyplacophoran species in which high temperatures and high tides influence reproductive timing, while an increase in daylight boosts gonad development.

**Key words:** Gonad, environmental factors, Polyplacophora, length at first maturity

## INTRODUCTION

Two polyplacophoran species, *Chiton articulatus* (Sowerby in Broderip and Sowerby, 1832) and *Stenoplax magdalenensis* (Hinds, 1845), are exploited intensively and with no restriction throughout their distribution range along the Mexican Pacific coast for use as human food, as bait for lobster fishing, and for manufacturing handicrafts (Ríos-Jara *et al.* 2006). *Chiton virgulatus* Sowerby II, 1840 is a species endemic to the coasts of the Gulf of California (Gonzalez 1993), for which no studies addressing its abundance are available. However, this species may be a resource with fishery potential.

The existing studies on *C. virgulatus* describe the oogenesis of this species considering three oocyte development stages (previtellogenic oocyte, immature vitellogenic oocyte, and mature vitellogenic oocyte) (Vélez-Arellano *et al.* 2014). Besides, some studies also describe the diversity of diatoms in the diet of *C. virgulatus* and conclude that diatoms are not the only food source for this species (Siqueiros-Beltrones & Argumedo-Hernandez 2012). Studies on the reproductive cycle of *C. articulatus* in Mexico are scarce (Rojas-Herrera 1988, Holguin-Quiñones & Michel-Morfín 2002, Flores-Campaña *et al.* 2007, García-Ibáñez *et al.* 2013, Morfín-Jiménez *et al.* 2014, Ramírez-Álvarez *et al.* 2014).

The proper management of a fishery resource, whether overexploited, under exploitation, or with exploitation potential, demands a thorough knowledge of its reproductive cycle, as this allows identifying the spawning season, estimating the size at reproductive recruitment, and setting a minimum catch size (Baqueiro *et al.* 1992). In studying the reproduction of many marine invertebrates, the histological examination of gonadal tissue is the most reliable method to recognize seasonal gonadal changes and determine the frequency and duration of spawning in a natural population (Jaramillo & Navarro 1995, Barón & Ciocco 2001). Other approaches for examining the reproductive cycle rely on calculating gonad, digestive gland and muscle performance indices (Villalejo-Fuerte & Ceballos-Vázquez 1996) and analyzing the monthly average diameter of oocytes (Grant & Tyler 1983).

A number of studies have described the reproductive cycle of various polyplacophoran species, including *Katharina tunicata* (Wood, 1815) (Giese *et al.* 1959, Himmelman 1978), *Mopalia hindsi* (Sowerby MS, Reeve, 1847) (Giese *et al.* 1959), *Acanthopleura japonica* (Lyschke, 1873) (Yoshioka 1987a, b), *Chiton cumingsii* Frembly, 1827 (Ishiyama 1995), *Acanthopleura gemmata* (Blainville, 1825) (Soliman *et al.* 1996, Barbosa *et al.* 2009), and several *Lepidochitona* species: *L. dentiens* (Gould, 1846), *L. hartwegii* (Carpenter, 1855), *L. berrryana* Eernisse, 1986, *L. thomasi* (Pilsbry, 1898), *L. caverna* Eernisse, 1986, and *L. fernaldi* Eernisse, 1986 (Eernisse 1988).

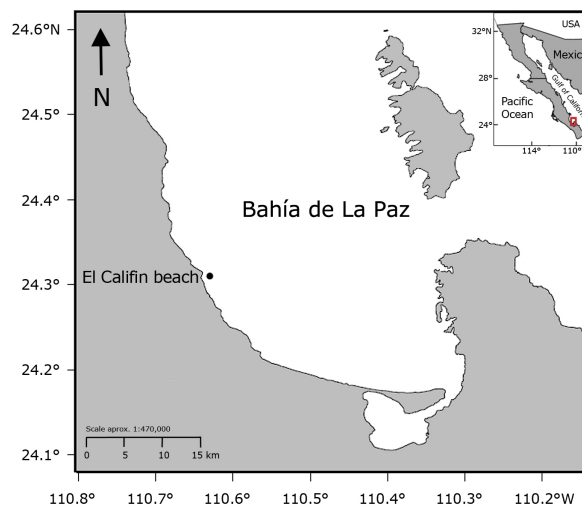
The reproductive cycles of marine invertebrates are usually determined by interactions between exogenous (*e.g.*, photoperiod, tidal level, and temperature) (Rose *et al.* 1990), as well as by endogenous factors (*e.g.*, endocrine and neuronal) (López *et al.* 2005). Thus, reproductive periodicity in these organisms is regulated not only by the genome but also by variations in abiotic factors (Mackie 1984) acting as environmental signals that trigger gonadal maturation. This is particularly the case in intertidal zones inhabited by chitons, which are ecosystems permanently subjected to environmental fluctuations (Sakker 1986, Yoshioka 1987a). Reproductive cycles can be annual, biannual, seasonal, or continuous, depending on the particular locality and species (Sastry 1979).

The reproductive cycles of polyplacophoran species inhabiting Mexican coasts have been little investigated to date; the only species studied so far is *Chiton articulatus* (Avila-Poveda 2013, Morfin-Jiménez *et al.* 2014, Ramírez-Álvarez *et al.* 2014, Abadía-Chanona 2015). In this study, the reproductive cycle and the size at the first maturity of *C. virgulatus* were determined along with establishing its relationship with air and sea surface temperature, photoperiod, and tides in Bahía de La Paz, Gulf of California, México.

## MATERIALS AND METHODS

The sampling area was Bahía de La Paz, Baja California Sur, Mexico, located at 24.1-24.8°N and 110.2-110.8°W. This is the largest bay on the eastern coast of the Baja California peninsula. *C. virgulatus* specimens were harvested monthly in the intertidal zone of the rocky beach El Califin (24°19'16"N, 110°38'27"W) (Fig. 1) from September 2008 to September 2009. Averages of 25 specimens were monthly and randomly collected. Body mass and total length of the specimens collected was recorded. All specimens were deshelled and dissected to remove the gonad; the gonad weight was recorded. Conventional histological analysis was done following gonad immersion in formalin (10%) prepared in filtered seawater for 48 h, then dehydrated and embedded in paraffin. Several 5 µm-thick sections were obtained and stained using Harris' hematoxylin-eosin and Masson's trichrome techniques (Luna 1968). Specimens were sexed by examining photomicrographs of slides captured with a Leica DMLS. Based on the study by Barbosa *et al.* (2009) and the morphological characteristics observed, the gonadal cycle of *C. virgulatus* was divided into four stages (undifferentiated, developing, mature and spawning).

The diameter of 100 oocytes selected at random from each gonad of developing or mature females were measured according to Grant & Tyler (1983), using the software Sigma Scan Pro Version 5 (Systat Software, Inc., USA).



**Figure 1. Study area: El Califin beach, Bahía de La Paz, Gulf of California, Mexico / Área de estudio: Playa El Califín, Bahía de La Paz, Golfo de California, México**

Length at first maturity (L50) is defined as the one at which 50% of all organisms are sexually mature (Somerton 1980). In order to, the frequency of mature and spawning organisms was obtained considering 2 mm size intervals. The relative frequency and cumulative relative frequency were calculated to define the range of length at first maturity, using the following equation:

$$Y = \frac{1}{1 + e^{-(a + bLt)}}$$

Where  $Y$  is the proportion of mature and spawning organisms;  $Lt$  is total length (mm); and  $a$  and  $b$  are constants.

The gonad index (GI) was calculated as defined by Villalejo-Fuerte & Ceballos-Vázquez (1996), by dividing gonad weight by total weight; the gonad index was an indicator of the complete reproductive cycle.

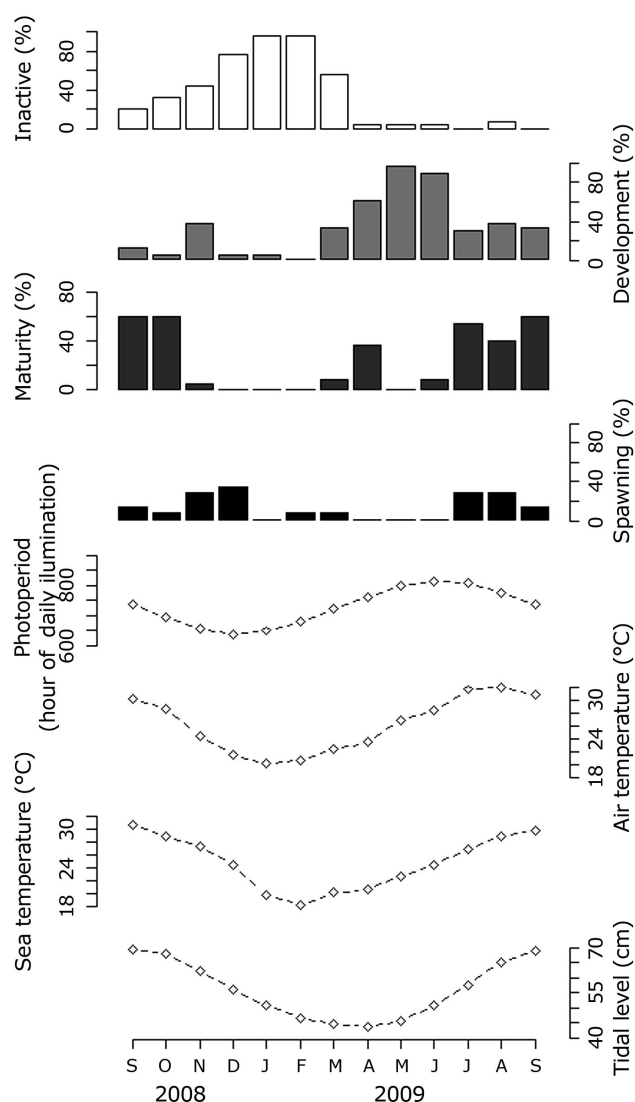
Sea surface temperature (SST) was recorded at the time of harvest with a mercury thermometer; air temperature (AT) data were obtained from the meteorological station at La Paz city; photoperiod and tidal cycle (TC) data were obtained from the MARv09 program provided by CICESE in Ensenada, Baja California, Mexico (CICESE)<sup>1</sup>.

The correlations between the indices, gonad stage, and environmental variables (SST, AT, CM) were examined with the Spearman's rank correlation coefficient (Spearman 1904), and statistical significance was evaluated with the Student's  $t$ -test for  $n$  (number of data) and  $n-2$  degrees of freedom (Zar 2010). A chi-square test showed the significant difference in the sex ratio ( $P > 0.05$ ). Monthly variations in oocyte diameter and GI were analyzed through a non-parametric test (Kruskal-Wallis test). All the statistical tests were run using the Statistica 8.0 software (StatSoft Inc., USA).

## RESULTS

### ENVIRONMENTAL PARAMETERS

Monthly SST, AT, photoperiod, and tidal cycle data are shown in Figure 2. SST maximum temperature was recorded in September 2008 (30.6 °C) and decreased steadily until February 2009 (18.1 °C). The highest AT was recorded in September 2008 (29.9 °C), decreasing gradually to 19.9 °C in January 2009, and then rising steadily again to reach its highest value, 31.8 °C, in August 2009. As for photoperiod, the longest days were recorded in June and July, and the shortest in December and January. The tide cycle showed its highest values in September 2008 and 2009 (68.9 and 68.5 cm, respectively); it dropped gradually from September 2008 to April 2009 (43.23 cm) and then rose again until September 2009.



**Figure 2.** Frequency of occurrence of the reproductive stages of *Chiton virgulatus* and fluctuation in environmental parameters in Bahía de La Paz, Mexico / Frecuencia de las etapas del ciclo reproductivo de *Chiton virgulatus* y variaciones ambientales en Bahía de La Paz, México

<sup>1</sup><http://predmar.cicese.mx/programa/>

A total of 325 *C. virgulatus* specimens were collected. Shell length oscillated between 24 to 46 mm, with a mean total length of  $32.18 \pm 4$  mm. Of these specimens, 112 were sexually undifferentiated, and the  $\chi^2$  test showed that the sex ratio did not deviate significantly from 1:1.

The gonad of *C. virgulatus* is cylindrical, ranging in color from green to brown in females and from beige to pink in males, with the shade varying in relation to the degree of maturity.

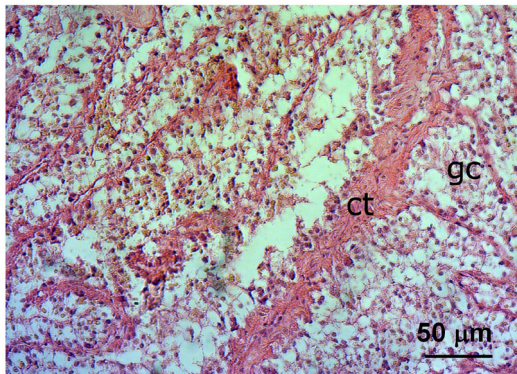
Histologically, in both sexes the gonad is surrounded by connective tissue with invaginations (sex cords) projecting towards the gonad center; sex cords show a radial arrangement within the gonad. The connective tissue gives rise to germinal tissue from which germ cells originate; fully developed germ cells detach and gametes accumulate in the center of the gonad.

#### UNDIFFERENTIATED STAGE

No sex cells can be observed at this stage; only connective tissue is apparent (Fig. 3).

#### DEVELOPING STAGE

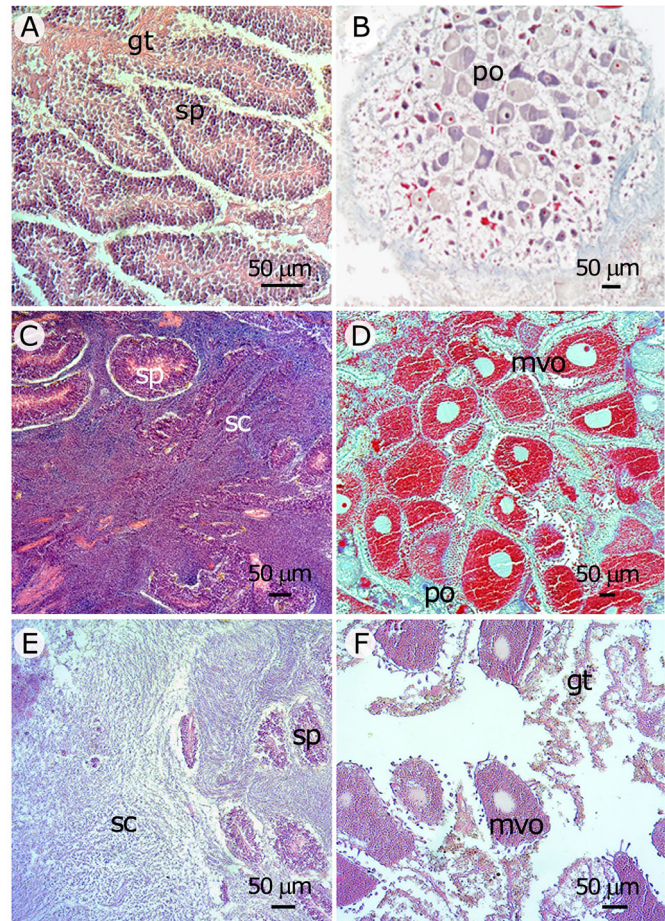
Males typically show numerous spermatogonia, spermatocytes, and spermatids growing along sex cords towards the lumen of the gonad, where some sperm cells can be found (Fig. 4A). In females, previtellogenic oocytes and some immature vitellogenic oocytes predominate, among which empty spaces can be observed (Fig. 4B).



**Figure 3.** Histological section of undifferentiated gonads of *Chiton virgulatus* in Bahía de La Paz, Mexico. Connective tissue (ct), germ cells (gc). Hematoxylin-eosin staining. Bar 50  $\mu$ m / Corte histológico de gónada indiferenciada de *Chiton virgulatus* en Bahía de La Paz, México. Tejido conectivo (ct), células germinales (gc). Tinción hematoxilina-eosina. Barra 50  $\mu$ m

#### MATURE STAGE

Male gonads characteristically show a reduced number of spermatocytes and spermatogonies along the sex cords but contain numerous sperm cells (Fig. 4C). In female gonads, mature vitellogenic oocytes predominate, the number of previtellogenic and immature vitellogenic oocytes has decreased, and the spaces between oocytes have disappeared as a result of oocyte growth (Fig. 4D).



**Figure 4.** Histological section of gonads of *Chiton virgulatus* in Bahía de La Paz, Mexico. A) male gonad in the developing stage. Hematoxylin-eosin staining; B) female gonad in the developing stage. Masson's trichrome staining; C) mature male gonad. Hematoxylin-eosin staining; D) mature female gonad. Masson's trichrome staining; E) spawning male gonad. Hematoxylin-eosin staining; F) spawning female gonad. Hematoxylin-eosin staining. Spermatocytes (sp), sperm cells (sc), mature vitellogenic oocytes (mvo), previtellogenic oocytes (po), germinal tissue (gt) / Corte histológico de la gónada de *Chiton virgulatus* en Bahía de La Paz, México. A) gónada de macho en etapa de desarrollo. Tinción hematoxilina-eosina; B) gónada de hembra en etapa de desarrollo. Tinción tricrómica de Masson; C) gónada madura de macho. Tinción hematoxilina-eosina; D) gónada madura de hembra. Tinción tricrómica de Masson; E) gónada de macho en desove. Tinción hematoxilina-eosina; F) gónada de hembra en desove. Tinción hematoxilina-eosina. Espermatocitos (sp), espermatozoides (sc), ovocito vitelogénico maduro (mvo), ovocito previtelogénico (po), tejido germinal (gt)

## SPAWNING STAGE

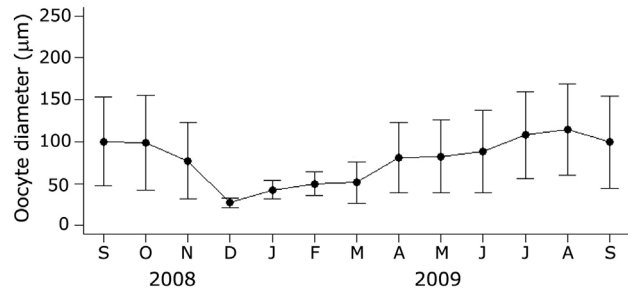
In males, empty spaces in gonad tissue and some free sperm cells can be observed (Fig. 4E). In females, large voids are present between vitellogenic oocytes; connective tissue trabeculae appear disarranged (Fig. 4F).

## REPRODUCTIVE CYCLE

The frequency of the reproductive stages is summarized in Figure 2. It shows that all stages occur throughout the year. The undifferentiated stage was more frequent in January and February (96%), and dropped when AT was high ( $R = -0.75$ ) and days were shorter ( $R = -0.83$ ). The developing stage occurred more frequently from April to June ( $\leq 60\%$ ). The maturity stage was observed mainly in the summer (July to October), correlated with high SST ( $R = 0.79$ ) and high AT ( $R = 0.8$ ), and when the tidal cycle shows higher values ( $R = 0.64$ ). The spawning stage also peaked in summer-fall, from September (8%) to December (20%); this stage coincides with high levels of the tidal cycle ( $R = 0.57$ ).

## OOCYTE DIAMETER

The mean oocyte diameter showed significant differences between months ( $P < 0.05$ ) (Table 1). The mean oocyte diameter was larger in September ( $100 \pm 52 \mu\text{m}$ ) and October ( $98 \pm 56 \mu\text{m}$ ) 2008, then decreased gradually to December 2008, when the smallest mean diameter was recorded ( $27 \pm 6 \mu\text{m}$ ). Oocyte size remained low for a few months but then increased steadily until April 2009 ( $81 \pm 41 \mu\text{m}$ ). The largest mean diameter was recorded in August 2009 ( $114 \pm 54 \mu\text{m}$ ) (Fig. 5).



**Figure 5.** Monthly variations in mean oocyte diameter of *Chiton virgulatus* in Bahía de La Paz, Mexico. Bars represent the standard deviation / Variación mensual en el diámetro promedio mensual de los ovocitos de *Chiton virgulatus* en Bahía de La Paz, México. Las barras muestran la desviación estándar

**Table 1.** Significance of the post-hoc (Mann-Whitney) test after the paired comparison (Kruskal-Wallis test) of monthly mean oocyte diameter / Valores de significancia en la prueba post-hoc (Mann-Whitney) después de la comparación pareada (prueba de Kruskal-Wallis) del diámetro promedio mensual de los ovocitos

	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
	2018				2019								
2018	Sep	1.000											
	Oct	1.000	1.000										
	Nov	0.221	0.463	1.000									
	Dec	0.0001*	0.0001*	0.005*	1.000								
2019	Jan	0.002*	0.004*	0.930	1.000	1.000							
	Feb	0.187	0.276	1.000	1.000	1.000	1.000						
	Mar	0.0001*	0.0001*	0.463	1.000	1.000	1.000	1.000					
	Apr	1.000	1.000	1.000	0.001*	0.255	1.000	0.039*	1.000				
	May	1.000	1.000	1.000	0.001*	0.202	1.000	0.026*	1.000	1.000			
	Jun	1.000	1.000	1.000	0.0001*	0.081	1.000	0.004*	1.000	1.000	1.000		
	Jul	1.000	1.000	0.016	0.0001*	0.000*	0.047*	0.0001*	0.122	0.209	0.941	1.00	
	Aug	1.000	1.000	0.001	0.0001*	0.000*	0.013*	0.0001*	0.010*	0.019*	0.110	1.00	1.00
	Sep	1.000	1.000	0.439	0.0001*	0.004*	0.301	0.0001*	1.000	1.000	1.000	1.00	1.00

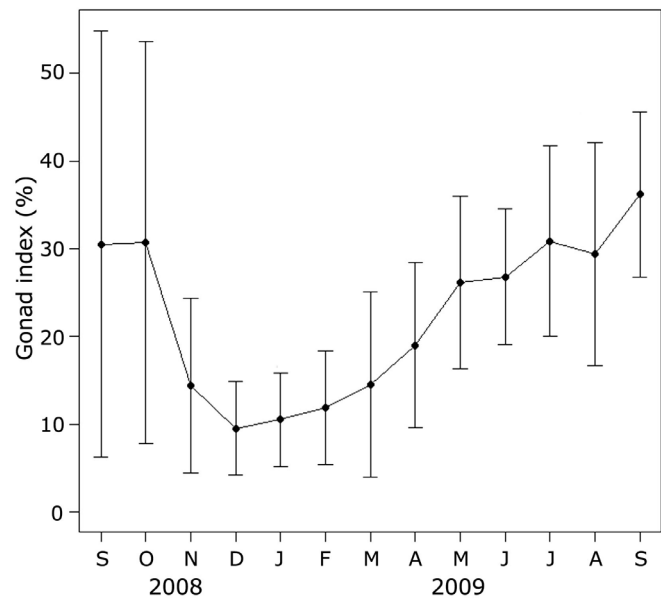
\* indicates significant differences

## GONAD INDEX

This index showed significant differences between months ( $P < 0.05$ ). Peak values were recorded in September and October 2008 (30.52 and 30.75%, respectively). The index decreased in November (14.4%) and remained low until April (19.0%), then increased gradually until September 2009 when it reached the peak value again (36.2%) (Table 2, Fig. 6).

## SIZE AT REPRODUCTIVE RECRUITMENT

The length of *C. virgulatus* ranged from 24 to 46 mm ( $32.18 \pm 4$  mm); the size at which at least 50% of the population is sexually mature was estimated at 31.42 mm in length. However, 24-mm specimens were observed that already showing signs of maturity (Fig. 7).



**Figure 6.** Monthly variations in gonad index values for *Chiton virgulatus* in Bahía de La Paz, Mexico. Bars represent the standard deviation / Variación mensual del valor del índice gonádico de *Chiton virgulatus* en Bahía de La Paz, México. Las barras muestran la desviación estándar

**Table 2.** Significance of the post-hoc (Mann-Whitney) test after the paired comparison (Kruskal-Wallis test) of gonad index per month / Valores de significancia en la prueba post-hoc (Mann-Whitney) después de la comparación pareada (prueba de Kruskal-Wallis) del índice gonádico por mes

	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	
	2018				2019									
2018	Sep	1.000												
	Oct	1.000	1.000											
	Nov	0.059	0.261	1.000										
	Dec	0.000*	0.002*	1.000	1.000									
2019	Jan	0.001*	0.009*	1.000	1.000	1.000								
	Feb	0.013*	0.069	1.000	1.000	1.000	1.000							
	Mar	0.124	0.501	1.000	1.000	1.000	1.000	1.000						
	Apr	1.000	1.000	1.000	0.39	1.000	1.000	1.000	1.000					
	May	1.000	1.000	0.078	0.001*	0.002*	0.018*	0.161	1.000	1.00				
	Jun	1.000	1.000	0.033*	0.001*	0.001*	0.007*	0.071	1.000	1.00	1.00			
	Jul	1.000	1.000	0.001*	0.001*	0.0001*	0.0001*	0.003*	0.470	1.00	1.00	1.00		
	Aug	1.000	1.000	0.008*	0.001*	0.0001*	0.002*	0.019*	1.000	1.00	1.00	1.00	1.00	
	Sep	1.000	0.822	0.0001*	0.001*	0.0001*	0.0001*	0.0001*	0.007*	1.00	1.00	1.00	1.00	1.00

\* indicates significant differences

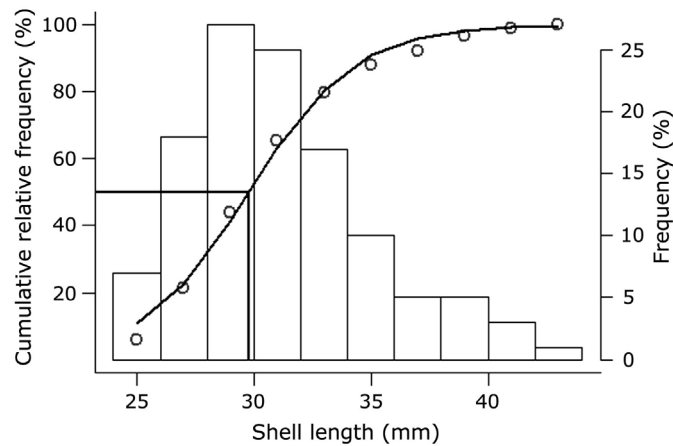


Figure 7. Size at reproductive recruitment in *Chiton virgulatus* in Bahía de La Paz, Mexico. September 2008-September 2009 / Longitud de reclutamiento a la reproducción de *Chiton virgulatus* en Bahía de La Paz, México. Septiembre 2008-Septiembre 2009

## DISCUSSION

A feature of the Polyplacophora that allows differentiating males from females is gonad coloration. In males, mature gonads range from brown-red to orange through pink-salmon, while immature males feature white gonads. In females, female gonads display shades ranging from dark green to black (Glynn 1970, Pearse 1979, Rojas-Herrera 1988). In *C. virgulatus*, female gonads are brown-colored when immature and turn green as they mature; male gonads are white when immature and become pink at maturity. The anatomical structure of the gonad showed features similar to those already described for other chiton species, including *Onithochiton quercinus*, *Chiton pelliserpentis*, *Plaxiphora paeteliana*, *C. articulatus*, and *Chiton albolineatus* (Sakker 1984, Avila-Poveda & Abadía-Chanona 2013).

The sex ratio was 1:1 throughout the year. This differs from what has been reported for other polyplacophoran species, including *Acanthopleura granulata* and *Chiton tuberculatus* (Glynn 1970), *Chiton cumingsii* (Ishiyama 1995, Sotil 2004), and *A. gemmata* (Barbosa *et al.* 2009), in which the sex ratio is biased towards males. In this regard, Sotil (2004) pointed out that given the dispersal of sperm caused by sea turbulence, it is likely that the predominance of males in the population ensures oocyte fertilization by increasing the number of sperm cells in water. The 1:1 sex ratio observed in this *C. virgulatus* population suggests that it might have adopted other reproductive tactics for gamete dispersal, as suggested by the fact that it displays continuous spawning throughout the reproductive cycle.

*C. virgulatus* behaved like a partial spawner, a feature commonly observed in the class Polyplacophora, for example in *Acanthopleura japonica* (Yoshioka 1987a), *Ischnochiton australis*, and *Chiton cumingsii*.

*Chiton virgulatus* showed a reproductive peak in summer-fall (Fig. 3), similar to other chiton species, including *Mopalia hindsii* (Giese *et al.* 1959), *Acanthopleura granulata*, *Chiton tuberculatus* (Glynn 1970), *Ischnochiton australis*, and *Liolophura gaimardi* (Currier 1989). In winter-spring, a higher frequency of sexually undifferentiated organisms and a decrease in spawning frequency were observed. This might be related to the fact that the coastline where specimens were collected is influenced by bottom surges carrying a large amount of sediment (Cruz-Chávez 2008, Obeso-Nieblas *et al.* 2014). In Santa Monica Bay, USA, it was observed that *Mopalia muscosa* was not able to feed actively due to wave action and the presence of sand, which caused stress and led to gonad reabsorption (Booolootian 1965). The high frequency of undifferentiated organisms during the fall and spring could also be due to a conservative tactic, as observed for other molluscs such as *Spondylus calcifer* (Villalejo-Fuerte *et al.* 2002), *Megapitaria squalida* (Arellano-Martinez *et al.* 2006), and *Panopea globosa* (Aragón-Noriega *et al.* 2007). These species accumulate energy during part of the year; when favorable conditions occur (*i.e.*, high temperature, trigger gonad development, and maturity).

Reproductive cycles of marine invertebrates are commonly governed by interactions between exogenous factors such as photoperiod, tide level, and temperature, which act as environmental signals that trigger gonadal maturation in species such as *Acanthopleura japonica* (Yoshioka 1987a), *Katherina tunicata* (Himmelman 1978), *Chiton cumingsii* (Ishiyama 1995), *Acanthopleura gemmata* (Barbosa *et al.* 2009), and several other molluscs. In the present study, mature specimens of *Chiton virgulatus* were also determined to be more common on El Califin beach when SST and AT were high (29.9 and 30.6 °C, respectively).

Similar to other chiton species such as *A. japonica* (Yoshioka 1987a), *A. granulata*, and *C. tuberculatus* (Glynn 1970), spawning of *C. virgulatus* occurs during the highest tides. Previous studies on *Katharina tunicata* and *Mopalia hindisii* in Santa Monica Bay, USA (Giese *et al.* 1959), and *C. articulatus* in Acapulco Bay, Guerrero, Mexico (Ramírez-Álvarez 2013) found that reproductive cycles are influenced by several local factors. Similarly, Himmelman (1978) found that the reproductive cycle of *Katherina tunicata* was not clearly related to temperature, photoperiod, lunar cycle, or tidal cycle. A probable explanation is that if *C. virgulatus* inhabits the intertidal zone, the populations may be affected by sea and air temperature as well as by the tidal cycle, being highly variable, not only over the annual cycle but also throughout the day.

The gonad development stage was directly affected by photoperiod. With shorter days, the frequency of sexually undifferentiated specimens increased and the frequency of developing organisms decreased. Although this relationship has not been reported for other chiton species, longer days have been found to trigger gonad development in invertebrates such as the starfish *Pisaster ochraceus* (Pearse & Eernisse 1982), molluscs such as *Octopus vulgaris* (Numata & Udaka 2009), and the bivalve *Solen marginatus* (López *et al.* 2005). In this regard, López *et al.* (2005) found a close relationship between the gametogenic process, reserve buildup, and food availability in water. The authors suggested that the high energy demand during reproduction is met by higher food intake, as a rise in photoperiod leads to an increase in organic matter availability. Therefore, these factors would be key for gonad maturation and spawning in this species.

This study shows that the gonad index is a good indicator of the different reproductive stages in *C. virgulatus*; this index has also been useful in *C. articulatus* (Ramírez-Álvarez 2013). On the other hand, tracking the monthly mean oocyte diameter was not useful for this purpose, as those values showed no apparent correlation with the reproductive stages, likely due to the co-occurrence of oocytes and other development stages in the same gonad, as described by Vélez-Arellano *et al.* (2014).

Specimens collected at El Califin beach were small (mean total length= 32.18 mm), compared to the reports of Abbott (1974) of 50-70 mm as maximum length. This might be due to hydrographic features, as El Califin beach is exposed to waves of moderate energy that cause a relatively intense abrasion of the rock surface, thus reducing the amount of biofilm and algae that can be used as food by chitons. Having more food available, chitons can obtain the energy required to attain larger sizes and more frequent spawning events. However, Otaíza (1986), found larger chitons most exposed to waves. *Chiton virgulatus* is a polyplacophoran in which temperature and high tide influence the reproductive cycle, while increased daylight triggers gonad development.

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