

Reproductive Cycle of *Hexaplex princeps* (Broderip, 1833)

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Abstract

During two annual periods, the reproductive cycle of the gastropod *Hexaplex princeps* from Puerto Ángel, Oaxaca, Mexico was studied through gonadal histology. Sex proportion for the collected individuals was not statistically different from parity although most of the time, the number of males was slightly larger than that of females, which only outnumbered males during the spawning season. The maturity stages established for females were: (1) initial oogenesis, (2) previtellogenic maturity, (3) vitellogenic maturity, (4) maturity, (5) spawning, and (6) resting; and for males: (1) initial spermatogenesis, (2) maturity, (3) spawning, (4) onset of the rest, and (5) resting. Monthly variations of maturation stages showed that *H. princeps* has an annual reproductive cycle with a long period of gonadal activity. The spawning season comprised from November (females) and December (males) to March, with activity peaks in January. From March to October (females) and from May to June (males), reproduction resting occurred. Spawning was related to high chlorophyll concentrations due to the upwelling processes resulting from the winds and to the cooler sea surface temperatures occurring from November to March. This study provides baseline information that may serve to establish measures for sustainable exploitation strategies and for future aquaculture implementation of this species.

Keywords: *Hexaplex princeps*, reproductive cycle, sexual proportion, histological analysis, maturity scales

1. Introduction

Marine mollusks constitute one of the more important world fisheries representing around 10% of the total value and quantity [1]. These invertebrates have been exploited since ancient times. Recently, it has been reported that omega fatty acids, including docosahexaenoic acid (DHA) are key to brain health and most likely helped to drive the evolution of the modern human brain, when hominin ancestors consumed rich DHA marine shellfish [2]. In the world, approximately 720 gastropod species are exploited [3, 4]. In Mexico, the gastropod catch in 2013 has the 19th place with 6011 ton [5].

The gastropod *Hexaplex princeps* has a spiny shell, height from 7.6 to 15.2 cm and whirl count 6 or 9; edge of lip armed with long, hollow, and frond-like spines;

is gonochoristic and the gonad with the digestive gland occupies the visceral coils [6]; distributes from the Gulf of California to Peru inhabiting moderately shallow waters [7, 8]. The presence of this species has been reported in Panama [9]. *Hexaplex princeps* (Broderip, 1833) is captured by several artisanal fisheries of Mexico: because of its size and taste, *H. princeps* is observed with less frequency in the subtidal zone at the Tenacatita (Jalisco) coral reef in search of other prey gastropods, although can be found in rocky substrates with heavy wave action [10]. At Acapulco (Guerrero), *H. princeps* is the second major exploited species after the oyster *Striostrea prismatica* [11]. Our observations along the study period suggest that at Puerto Ángel, Oaxaca, this species is an important fishery resource as it supports around 80% of the gastropod catch for local population and tourism consumption.

In the world, artisanal fisheries are in continuous expansion due to the growing demand and increasing value of appreciated species, and thus, the fisheries effort is augmenting [12]. Under this scenario, it is advisable (if not indispensable) to gather baseline biological information that may be used to propose management measures promoting long-term sustainable resource exploitation [4].

The study of the reproductive processes in marine organisms is a fundamental biological aspect, which permits to understand their population dynamics [13]. The reproductive season is a crucial life history trait and the proper timing of breeding may be important to provide the offspring with favorable environmental conditions and to influence parental fitness [14, 15]. The analysis of the reproductive cycle of organisms permits to know the adequate moment and intensity of the capture to avoid the population depletion.

The reproduction of Muricidae members (at which *H. princeps* belongs) has been studied in several instances: the gonad cycle of *Bolinus brandaris* at the South of Portugal [16] and at the South of Tunisia [4]; in the Gulf of California, Mexico, observations on the “Black Murex” *Hexaplex nigritus* [17] and *Hexaplex erythrostomus* [18] similar oviposition. In spite of the importance of *H. princeps* as a fishery resource, there is no information on its reproductive cycle. This knowledge gap makes necessary to carry out studies on the biological cycle of this species in order to take adequate management decisions leading toward its sustainable exploitation and even its aquaculture in the future.

Thus, this study is aimed to investigate the reproductive cycle of *Hexaplex princeps*, considering sexual proportion, gonad maturation, spawning periods, and maturity stages variation in relation with the surface temperature and chlorophyll concentration along two annual periods at Puerto Ángel, Oaxaca, Mexico.

2. Materials and methods

The organisms were obtained from the artisanal fishery with (as possible) monthly periodicity during two annual periods from January 2014 to November 2015. The organisms were caught with the help of two local free divers and the captain of an 8 m length vessel with a 40 HP outboard motor at depths from 5 to 15 m in rocky coast localities at the vicinity of Puerto Ángel, Oaxaca, Mexico, between 9:00 and 12:00 h local time (**Table 1, Figure 1**). The collecting sites were determined each date according to the atmospheric and sea conditions as well as the diver’s knowledge on the species availability in the zone. Our aim was to have a representative number of specimens from the region to gather the histological information from reproductive organs and tissues.

From the caught organisms, 10–15 individuals in the interval from 8 to 12 cm in length (interval that contained more than 90% of the lengths, we collected since

Site	Latitude (N)	Longitude (W)
Punta Cometa (PC)	15° 39' 35.4"	96° 33' 16.5"
San Agustínillo (SA)	15° 39' 48.6"	96° 33' 01.0"
Playa Panteón (PP)	15° 39' 56.1"	96° 29' 27.1"
Aragón (Ar)	15° 39' 38.2"	96° 31' 46.8"
Estacahuite (Es)	15° 40' 04.7"	96° 28' 54.5"
Bajos de Aceite (BA)	15° 40' 10.6"	96° 28' 29.6"
La Mina (LM)	15° 40' 26.7"	96° 28' 35.7"
La Boquilla (LB)	15° 40' 48.3"	96° 27' 58.4"
Secretario (Sr)	15° 41' 02.3"	96° 27' 00.5"
Tijera (Tj)	15° 41' 20.2"	96° 26' 26.3"
Dominguillo (Dm)	15° 41' 35.0"	96° 26' 02.2"
Playita, Tembo (PT)	15° 41' 36.1"	96° 25' 54.3"
Temperature (SST) and chlorophyll (CL)	15° 38' 44.9"	96° 28' 45.0"

Table 1.
 Specimen collection and environmental variables (surface sea temperature and chlorophyll) measure site geositions.

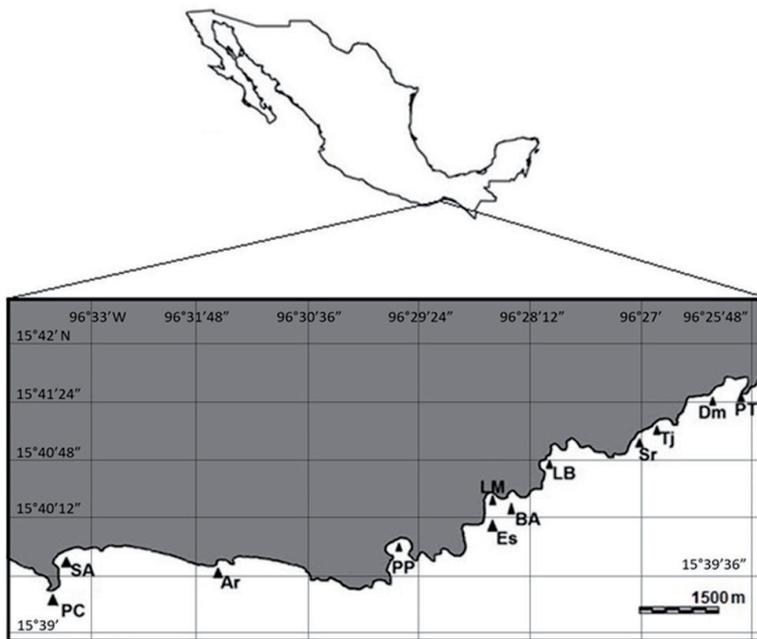


Figure 1.
 Geographical location of the study area, Puerto Ángel, Oaxaca, Mexico. The collection sites are indicated: PC: Punta Cometa, SA: San Agustínillo, Ar: Aragón, PP: Playa Panteón, ES: Estacahuite. LM: La Mina, BA: Bajos de Aceite, LB: La Boquilla, Sr: Secretario, Tj: Tijera, Dm: Dominguillo, PT: Playita Tembo.

2012) were separated and their shell broken to extract the soft parts, which were fixed in formalin 10% prepared with seawater [19]. Once fixed, the specimens were transported to the Biometry and Fisheries Biology Laboratory of the Facultad de Estudios Superiores Zaragoza, UNAM, where after 48 h were washed with tap water and preserved in 70% ethylic alcohol. As there are no external characters to

distinguish sex, the specimens were dissected to examine and search for the presence or absence of penis.

The sexual proportion was analyzed by means of the chi-squared goodness of fit test following the corrected Yates expression [20, 21].

The histological sections were carried out at the Histology Laboratory from the Morphology Department at the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. The alcohol-preserved specimens were dehydrated following the usual alcohol series (70–100%) and cleared in xylol before being included in paraplast and paraffin. The embedded tissues were sliced into sections of 5 μm thickness using a microtome and mounted over glass slides. The preparations were stained with the Hematoxylin-Eosin method [22] to facilitate the determination of the gonad development stages. The sections were fixed with Entalan and covered with glass slips. Finally, the preparations were observed and photographed by means of an optical microscope with attached camera.

The sea surface temperature (SST) and chlorophyll *a* of Puerto Ángel data were consulted from the GES DISC-NASA database [23, 24]. The monthly values were taken from a site in the vicinities of the Puerto Angel Bay (**Table 1**). To assess the statistical significance of the relationships between the maturity stages and the mean values of temperature and chlorophyll, two procedures were employed. In the first place, in order to clarify the pattern showed by the gonad stage percentages, a nonlinear resistant smoothing procedure was applied. The preferred smoother was the 4253eh,twice, which combines the smooth result of even span running median smoothers (4,2), the resistance of odd running medians (5,3) with end point adjustment (e), the “Hanning” weighted mean smoother (h) and the “re-roughing” (twice) step [25, 26, 27, 28, 29–32, 33].

The comparisons of the resulting time series data were performed by means of the cross-correlation analysis [34, 35, 33] between the percentages of maturity stages against the temperature and chlorophyll values. Additional cross-correlation analyses among maturity stages were made.

3. Results

3.1 Reproductive cycle

In total, 232 males and 214 females were captured. The sexual proportions throughout the study are included in **Table 2**. From the 446 individuals, 250 were analyzed for recognition and characterization of the gonad stages considering the degree of development besides the occurrence and abundance of gametes. The different gonad development stages were classified as follows: for females, six stages were established (**Table 3, Figure 2**). Stage 1 (S1) initial oogenesis, stage 2 (S2) previtellogenic maturity, stage 3 (S3) vitellogenic maturity, stage 4 (S4) maturity, stage 5 (S5) spawning, and finally, stage 6 (S6) resting. For males, five stages were recognized (**Table 4, Figure 3**). Stage 1 (S1) initial spermatogenesis, stage 2 (S2) maturity, stage 3 (S3) expulsion, stage 4 (S4) onset of rest, and stage 5 (S5) resting.

Spawning (S5) females of *H. princeps* (**Figure 4**) presented large percentages during January (60%), March (67%), and November (67%) of 2014. On the other hand, it is noted that 100% of the resting stage (S6) was registered in July. The months with larger spawning (S5) percentages during 2015 were January (60%), March (80%), and November (75%) and the months with resting (S6) larger frequency values were April (75%) and July (75%).

Collecting data and site (main)	Total	Sexo		Male:female proportion	χ^2 (Yates)	P
		Males	Females			
24/Jan/14 (Es)	93	40	53	1.00:1.33	1.55	0.21
21/Feb/14 (Dm)	02	02	0	—:—	0.50	0.48
21/Mar/14 (Ar)	26	16	10	1.60:1.00	0.96	0.33
25/Apr/14 (PP)	60	33	27	1.22:1.00	0.42	0.52
23/May/14 (ML)	53	27	26	1.04:1.00	0.00	1.00
15/Aug/14 (PC)	42	25	17	1.47:1.00	1.17	0.28
24/Oct/14 (PC)	13	04	09	1.00:2.25	1.23	0.27
28/Nov/14 (PT)	57	32	25	1.28:1.00	0.63	0.43
21/Dec/14 (Es)	12	07	05	1.40:1.00	0.08	0.77
30/Jan/15 (PT)	08	03	05	1.00:1.67	0.13	0.72
27/Mar/15 (PP)	07	02	05	1.00:2.50	0.57	0.45
30/Apr/15 (PP)	08	05	03	1.67:1.00	0.13	0.72
15/May/15 (LM)	08	04	04	1.00:1.00	0.13	0.72
12/Jun/15 (SA)	11	06	05	1.20:1.00	0.00	1.00
15/Jul/15 (BA)	09	06	03	2.00:1.00	0.44	0.50
21/Aug/15 (PP)	09	05	04	1.25:1.00	0.00	1.00
25/Sep/15 (PP)	10	05	05	1.00:1.00	0.10	0.75
16/Oct/15 (PP)	08	04	04	1.00:1.00	0.13	0.72
27/Nov/15 (PP)	10	06	04	1.50:1.00	0.10	0.75
Total	446	232	214	1.08:1.00	0.65	0.42

Site abbreviations according to **Table 1**.

Table 2.

Number of individuals by collecting date and site (main); sexual proportion and its statistical significance are included (χ^2 with Yates correction).

Stage 1 initial oogenesis (S1)	Occurrence of developing ovogonia and oocytes, thick follicle walls
Stage 2 previtellogenic maturity (S2)	Oocytes full of yolk granules; in some oocytes, the nucleus and nucleolus are observed; follicles completely mature full of oocytes
Stage 3 vitellogenic maturity (S3)	Follicles with thin walls and developing oocytes; yolk granules are observed and yolk platelets appear
Stage 4 maturity (S4)	Follicles full of yolk granules and platelets; thin follicle walls
Stage 5 spawning (S5)	Light in the follicles is observed; follicles partially empty; follicle walls thin with some remnant oocytes
Stage 6 resting (S6)	Some resting follicles besides cells or phagocytes in thick follicle walls; conspicuous conjunctive tissue

Table 3.

Characterization of gonad development stages for females of *Hexaplex princeps*.

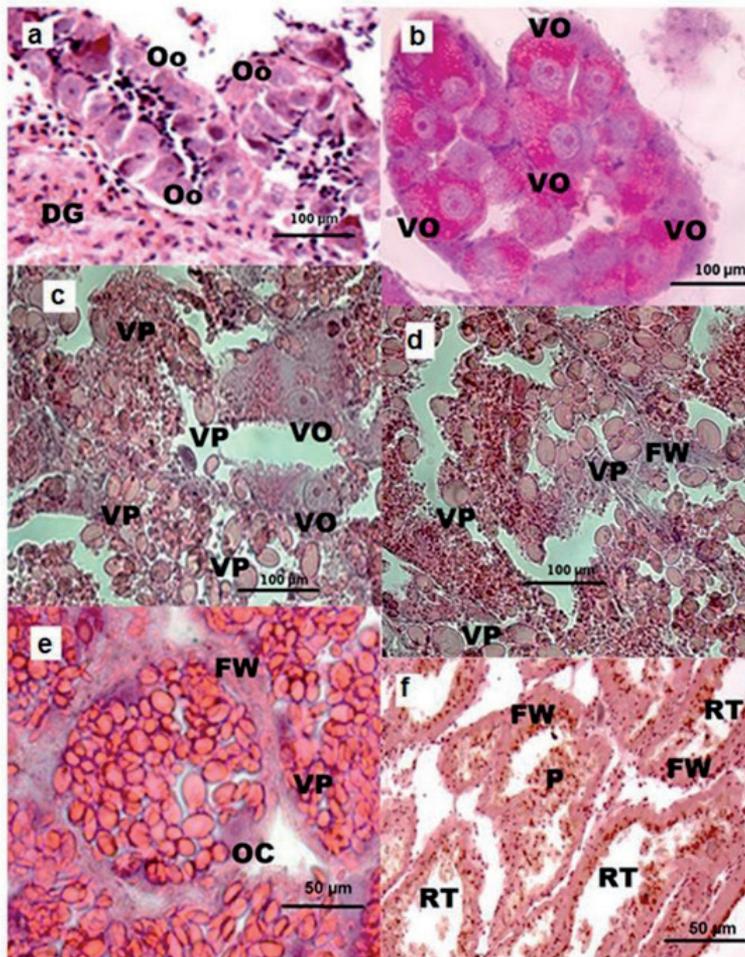


Figure 2. Histological sections of *Hexaplex princeps* females showing ovary stages. *a*: initial oogenesis (20X), *b*: previtellogenic mature (10X), *c*: vitellogenic mature (20X), *d*: mature (10X), *e*: spawning (10X), *f*: resting (10X). Oo: oogonia, Oc: oocytes, DG: digestive gland, CT: connective tissue, PV: vitelline platelets, PvO: previtellogenic oocytes, VO: vitellogenic oocytes, FW: follicular wall, RO: residual oocytes, P: phagocytes.

Males of *H. princeps* (**Figure 5**) presented spawning (S3) stage in January (100%) and March (50%); the reproductive resting stage (S5) occurred in

Stage 1 initial spermatogenesis (S1)	Follicles active, developed with immature cells; small separated follicles with numerous immature cells (spermatogonia and spermatocytes), thick follicle walls
Stage 2 maturity (S2)	Follicles utterly full with a greater quantity of spermatozooids, spermatogonia, spermatocytes, and spermatids
Stage 3 spawning (S3)	Mature spermatozooids in expulsion, ciliated cylindric epithelium with foldings
Stage 4 onset of rest (S4)	Some follicles in expulsion; empty and resting follicles are observed
Stage 5 resting (S5)	Empty follicle lumen; resting follicles due to the expulsion of spermatozooids are observed; conspicuous conjunctive tissue

Table 4. Characterization of gonad development stage for males of *Hexaplex princeps*.

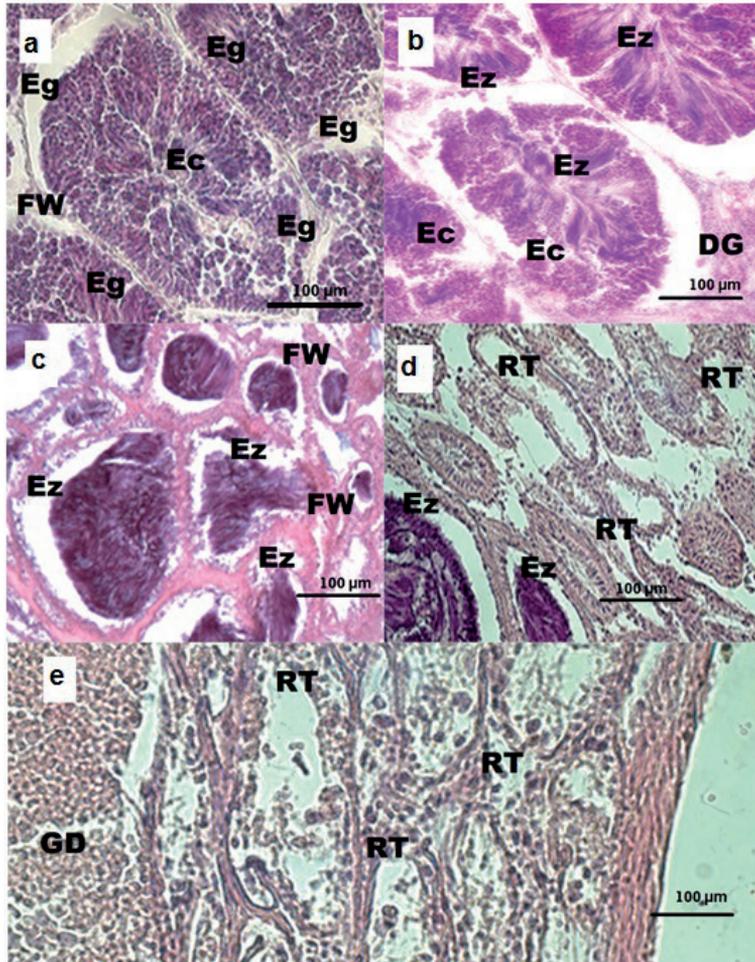


Figure 3. Histological sections of *Hexaplex princeps* males showing testis stages. *a*: initial spermatogenesis (50 µm), *b*: mature (100 µm), *c*: spawning (10x), *d*: onset of rest (100 µm), *e*: resting (50 µm). Eg: spermatogonia, Ec: spermatocytes, Ez: spermatozoa, RS: residual spermatozoa, DG: digestive gland, CT: connective tissue, P: phagocytes.

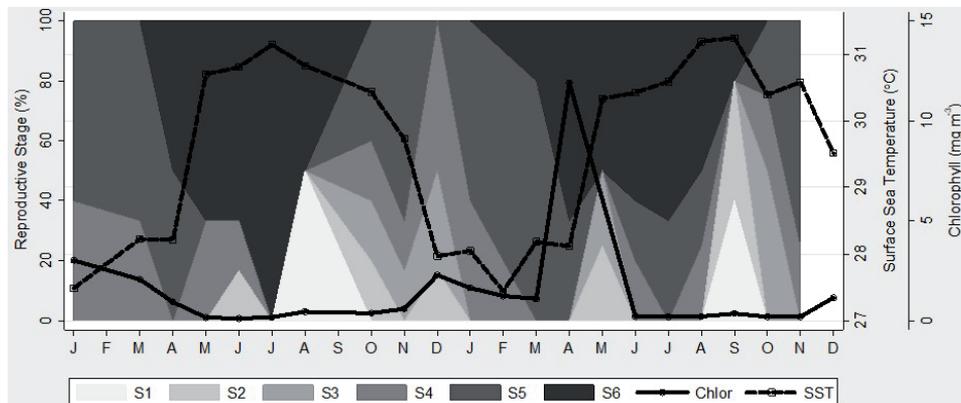


Figure 4. Gonad stage frequency 2014–2015 for *H. princeps* females by sampling date. Chlorophyll concentration (mg m⁻³) and Surface Water Temperature (°C) values are showed.

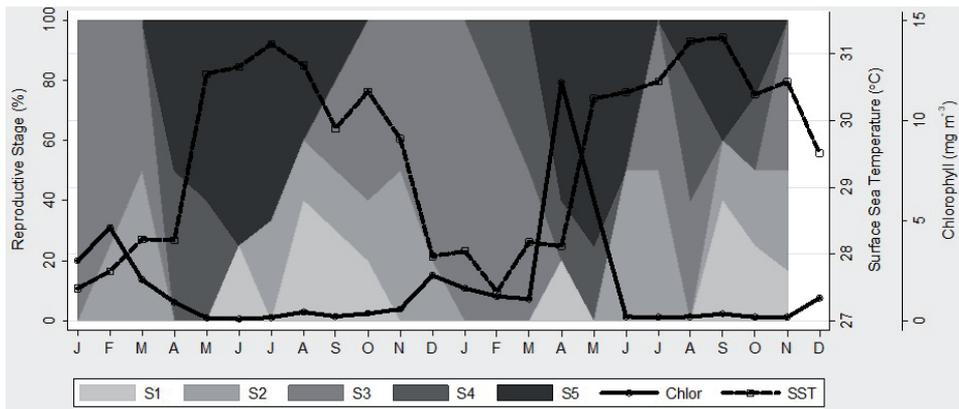


Figure 5. Gonad stage frequency 2014–2015 for *H. princeps* males by sampling date. Chlorophyll concentration (mg m^{-3}) and temperature ($^{\circ}\text{C}$) are included.

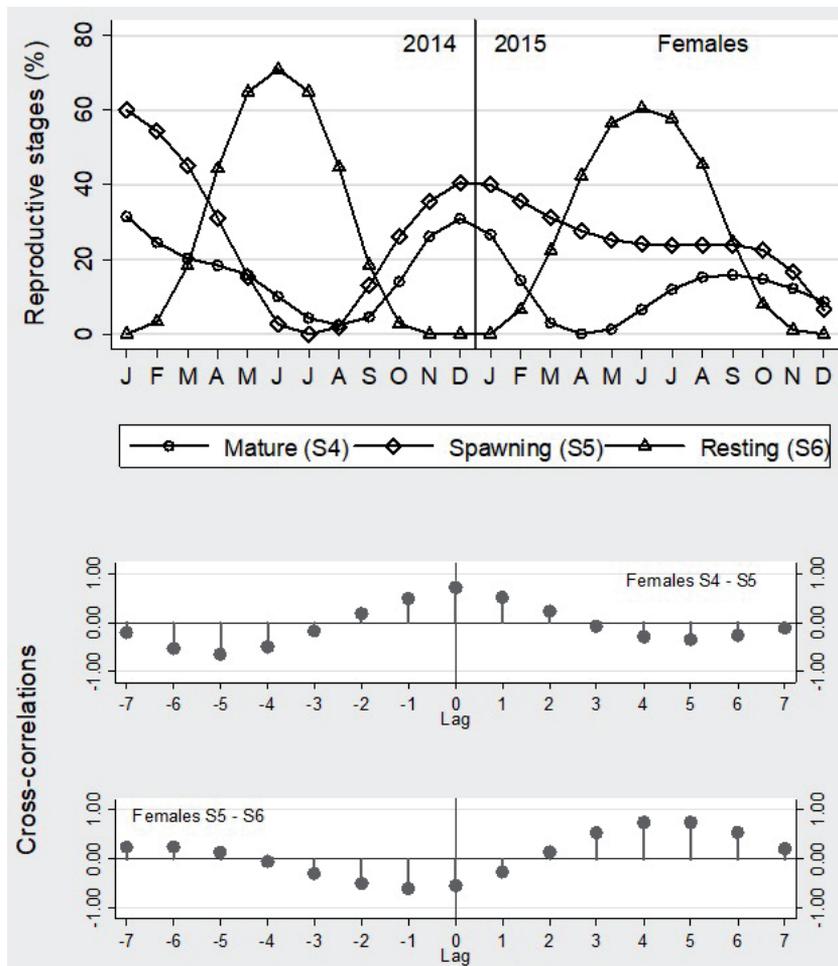


Figure 6. The smoothed frequency of the female reproductive stages along the study period and cross-correlation correlograms for mature-spawning (S4-S5) and spawning-resting (S5-S6) reproductive stages comparison.

May (60%), June (75%), and July (67%) 2014. The months of 2015 with larger spawning (S3) percentages were January (100%) and March (50%); finally, the months with the larger frequency of resting individuals (S5) were May (60%) and June (75%).

3.2 Chlorophyll *a* concentration and gonad cycle

In 2014, the highest chlorophyll *a* concentrations were observed in January (3.00 mg m^{-3}), February (4.00 mg m^{-3}), March (2.05 mg m^{-3}), and December (2.27 mg m^{-3}); in the same months, the larger frequency of individuals in the spawning stages (S5 females, S3 males) (Figures 4 and 5) was observed. For the same year, low chlorophyll concentrations were recorded in June (0.15 mg m^{-3}) and July (1.10 mg m^{-3}) months, which correspond with the larger frequency of females and males in the resting stages (S6 and S5, respectively) (Figures 4 and 5).

In 2015, the high chlorophyll concentrations were observed in January (1.62 mg m^{-3}), February (1.23 mg m^{-3}), March (1.08 mg m^{-3}), and (exceptionally) April (11.89 mg m^{-3}); except for April, in all other months, the stage with

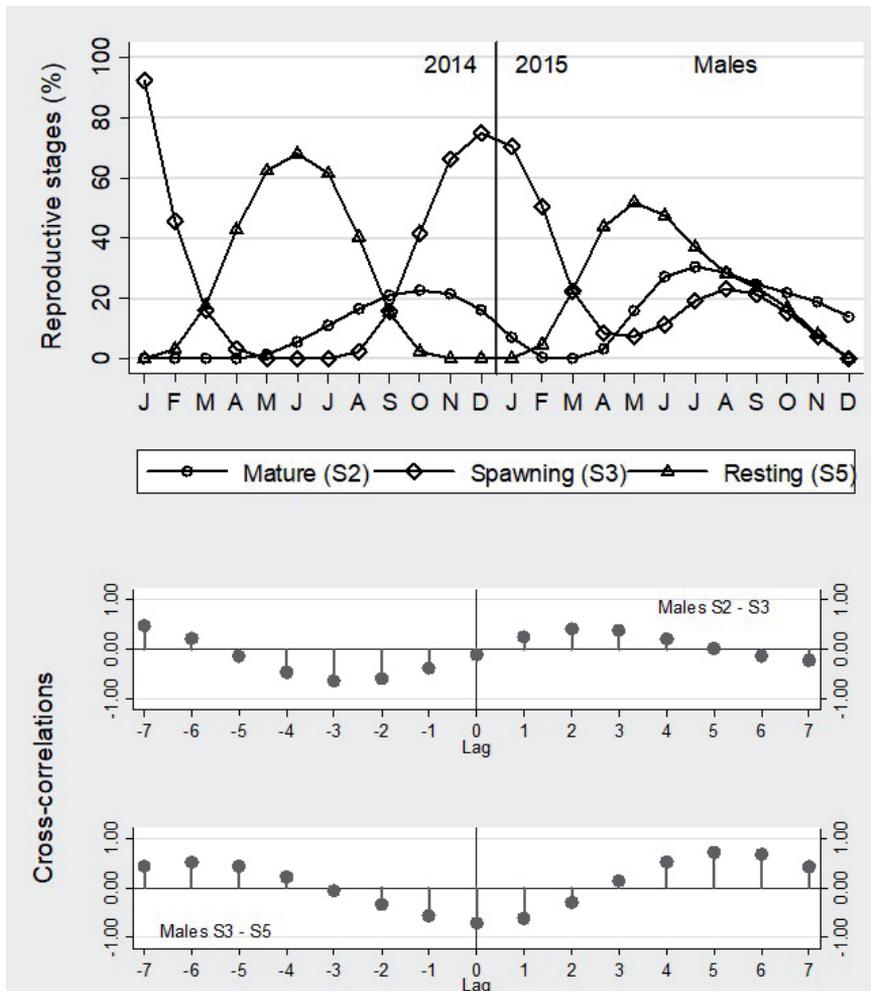


Figure 7. The smoothed frequency of the male reproductive stages along the study period and cross-correlation correlograms for mature-spawning (S2-S3) and spawning-resting (S3-S5) reproductive stages comparison.

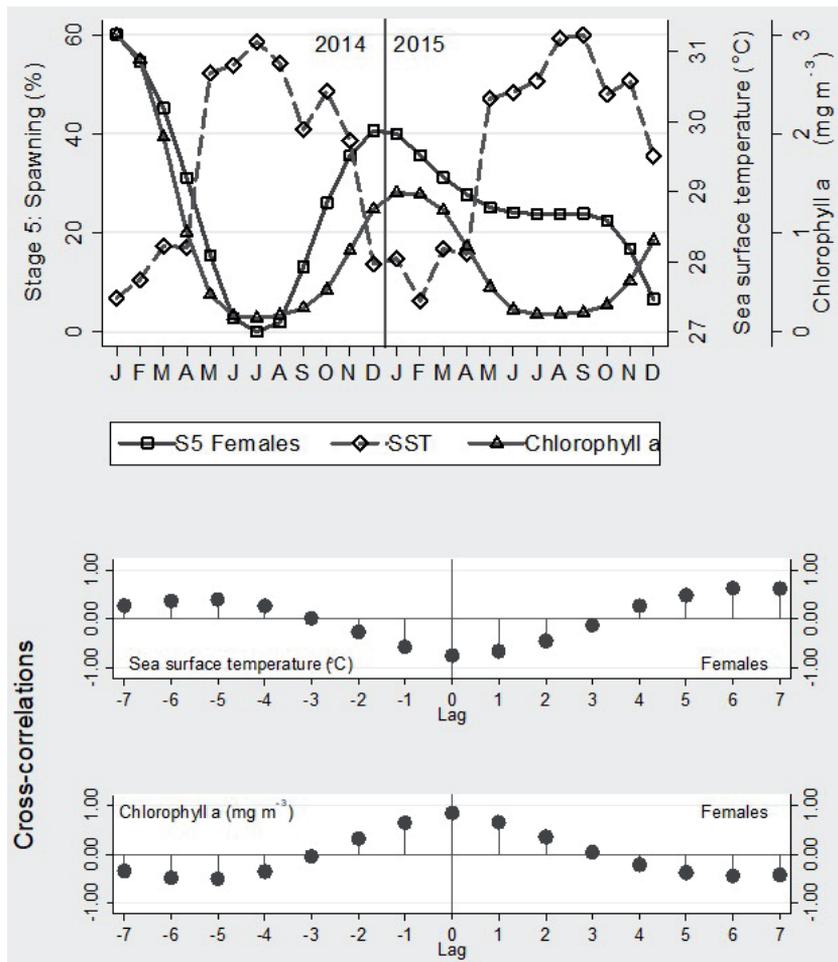


Figure 8.

The smoothed frequency of spawning females (S₅), surface sea temperature (SST) and chlorophyll concentration (CL) along the study period and cross-correlation correlograms for S₅-SST and S₅-CL comparison.

larger frequency was spawning (S₅ and S₃ for females and males, respectively) (Figures 4 and 5). In the same year, the lower chlorophyll concentrations occurred in July (0.18 mg m⁻³), October (0.18 mg m⁻³), and November (0.19 mg m⁻³). These chlorophyll concentration values were related to the resting stage of females and males (S₆ and S₅, respectively) (Figures 4 and 5).

It is possible that the April 2015, notably high (11.89 mg m⁻³), chlorophyll concentration originated a different pattern, in comparison with that from the same month of the previous year. The resting phase of females (S₆) and males (S₅) occurred with less frequency and the spawning gonad stage (S₅ females and S₃ males) extended to June, July, and August (Figures 4 and 5).

3.3 Temperature and gonad cycle

The lowest registered temperatures occurred in January (27.48°C), February (27.74°C), and December (27.96°C). In these months, it was observed that the spawning females (S₆) and males (S₃) were those with the highest frequency (Figures 4 and 5). The months with the highest temperatures were May (30.70°C),

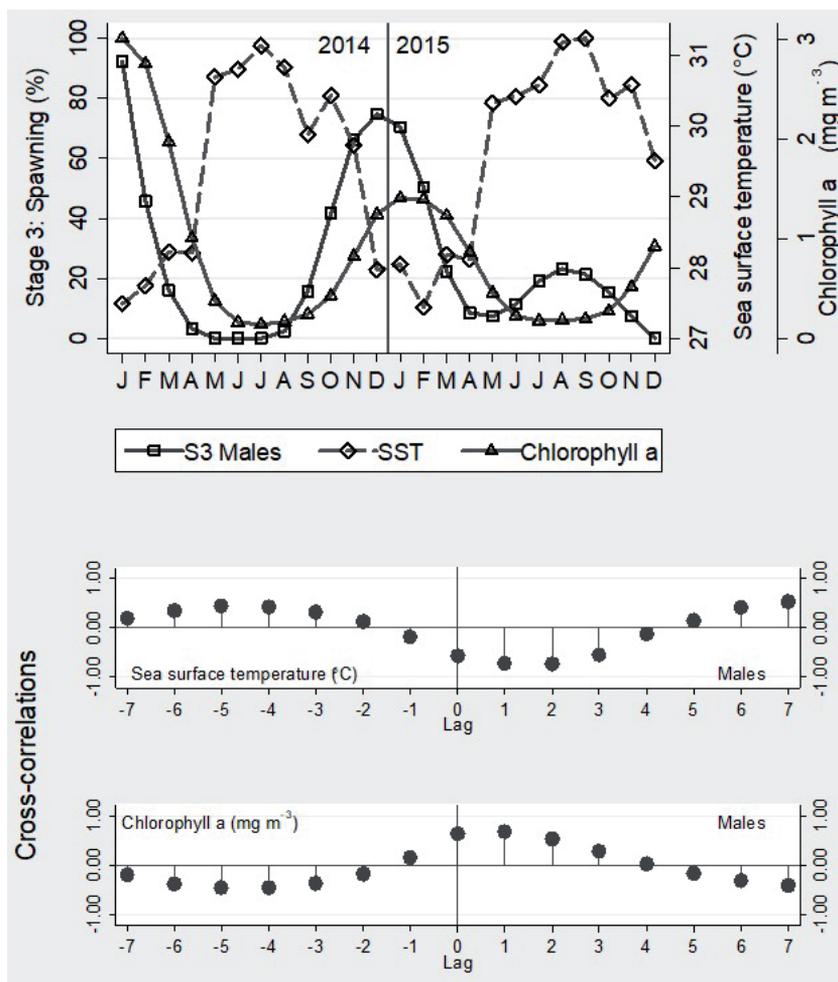


Figure 9. The smoothed frequency of spawning males (S_3), surface sea temperature (SST) and chlorophyll concentration (CL) along the study period and cross-correlation correlograms for S_3 -SST and S_3 -CL comparison.

June (31.14°C), and August (30.83°C), which were related to the larger frequency of the resting stages of females (S_6) and males (S_5) (Figures 4 and 5).

The lowest temperatures for 2015 were registered in January (28.04°C), February (27.44°C), and March (28.11°C), which corresponded with the highest frequencies of female and male in the spawning stage (S_5 and S_3 , respectively). The months with the larger temperature values were August (31.19°C) and September (31.24°C). In these months, female stage 4 (mature), 5 (spawning), and 6 (resting) were observed with 25, 25, and 50%, respectively; the male stages were 3 (spawning) and 4 (end of spawning, onset of the rest) (50 and 30%, respectively), and in September, 35% of stage 2 (maturity), 25% of phase 3 (spawning), and 40% of stage 5 (resting) were observed.

3.4 Smoothing and cross-correlation

To describe in more detail the variation and relationships of the reproduction stages and the environmental variables, sea surface temperature (SST) and chlorophyll *a* (Cl) concentrations, along the study period, the smoothed frequency of the reproductive stages were analyzed by cross-correlation. It can be easily seen

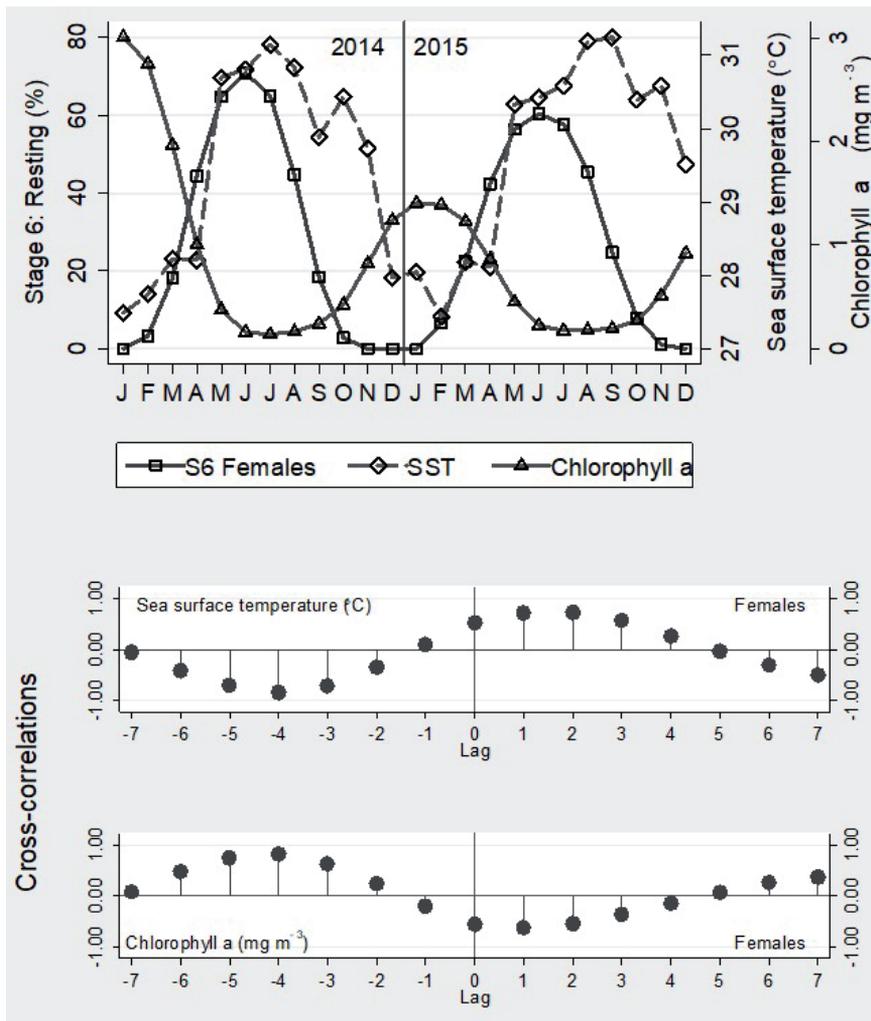


Figure 10. The smoothed frequency of resting females (S6), surface sea temperature (SST) and chlorophyll concentration (CL) along the study period and cross-correlation correlograms for S6-SST and S6-CL comparison.

(Figure 6) that during the cool months of the year (from October to February), the females mature (S4) and spawn (S5) from October to March. During 2015, the spawning period lasted longer than the previous year. Very clearly, the females are in reproductive rest (S6) during the warmer half of the year (from April to August). The cross-correlation of the series shows a significant positive trend between S4 and S5 stages synchronically and an inverse correlation with 4–5 months lag between spawning (S5) and rest (S6).

The males seem to mature (S2) early (July) but the trend is clear from September to January (Figure 7). The spawning males (S3) occur from October to February. Similarly than females, males attain the reproductive rest stage (S5) from April to August, during the warmer months.

The cross-correlograms show a direct relationship lagged 2 months between S2 and S3 (maturity, spawning stage) and a 6 month lagged high cross-correlation between spawning (S3) and resting (S5) males, corroborating significantly the above statements.

The spawning (S5) females showed a clear opposite (negative) correlation with the sea surface temperature values with a lag of 6 months, and concordant

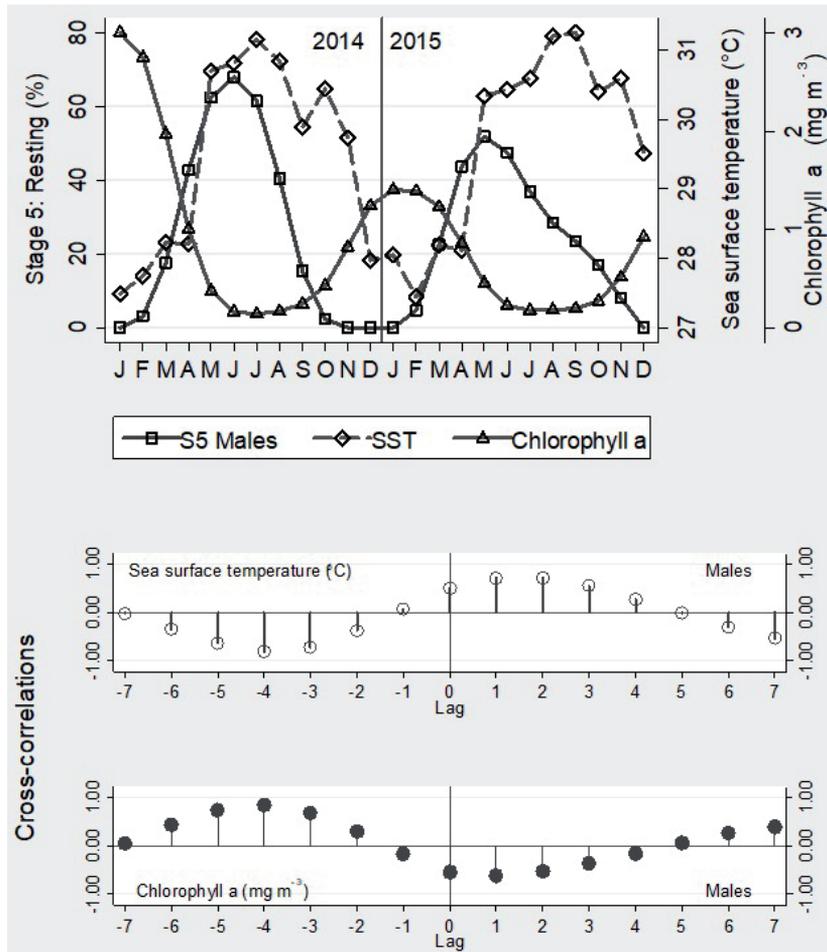


Figure 11. The smoothed frequency of resting males (S_5), surface sea temperature (SST) and chlorophyll concentration (CL) along the study period and cross-correlation correlograms for S_5 -SST and S_5 -CL comparison.

Sex	Sequence	Lag	Cross-correlation	P-value
Females	S4-S5	0	0.7144	0.0001
	S5-S6	4	0.7525	0.0000
Males	S2-S3	2	0.4165	0.0182
	S3-S5	5	0.7192	0.0000
Females	S5-SST	6	0.6278	0.0001
	S5-CL	0	0.8442	0.0000
Males	S3-SST	7	0.5366	0.0013
	S3-CL	1	0.6835	0.0000
Females	S6-SST	2	0.7281	0.0000
	S6-CL	-4	0.8384	0.0000
Males	S5-SST	2	0.7159	0.0000
	S5-CL	-4	0.8467	0.0000

Table 5. Cross-correlation analysis results resume: sex, sequences compared, time lag (month), cross-correlation, and significance values.

(positive) cross-correlation with chlorophyll *a* concentration with no lag (**Figure 8**). In **Figure 9**, it is possible to see that, as the females, spawning (S3) males had negative cross-correlation values with SST values (lagged around 7 months), and positive with chlorophyll (1-month lag). In contrast with the former trends, the resting females (S6) showed positive cross-correlations (lagged 2 months) with SST and negative relationship (with a lag of 7 months) with the chlorophyll concentrations (**Figure 10**). In a similar way as resting females, males in reproductive rest stage (S5) showed a direct trend with SST (lagged 1 or 2 months) and the opposite of the chlorophyll values (a lag of 7 months) (**Figure 11**). A resume of the cross-correlations significances is included in **Table 5**.

4. Discussion

The sex proportion of *H. princeps* from Puerto Ángel was found to be statistically balanced with a slight preponderance of males during the warmer months of the year. These findings correspond with the study of Vasconcelos *et al.* [36], who reported a balanced sex ratio and males dominating among smaller individuals of *Hexaplex (Trunculariopsis) trunculus* in the Ria Formosa Lagoon in Portugal. This is contrary to the unbalanced sex ratio reported by Elhasni *et al.* [4] for *Bolinus brandaris* (another Muricid) in Tunisia, where females surpassed males, mainly during the reproduction period. Although not significant, at Puerto Ángel in the cold months (January 2014 and January, March 2015), when the reproductive event occurs, the number of females was larger than that of males, which may be associated with the reproductive behavior of this species as the females tend to aggregate for oviposition.

The histological examination of the gonads of *Hexaplex princeps* at Puerto Ángel permitted to characterize six stages of maturity development in females (**Table 3**) and five for the males (**Table 4**). Although there are no previous reports on the histological maturity for *H. princeps*, the stages characterized in the present study for the females correspond closely to those suggested for *H. trunculus* from Portugal [36]. In the case of male maturity and based on our observations of the histological sections, we consider that only five stages are enough to describe the spermatogenic cycle.

Comparing both sexes, spawning and expulsion occurred in January, April, May, October, and November, and the larger frequency of resting individuals was registered in June and July. In this way, it is possible to recognize a period of spawning and expulsion from November to March with pikes in January and February. The resting period of females occurred from March to October, with peaks in July (2014) and April–July (2015), and the males presented high resting frequency values in June (2014) and May (2015). This does not correspond to the reproduction times reported for *H. erythrostomus* from Bahía Concepción, Baja California Sur, where the reproductive events were annotated during the warmer months (May–July) [37]; though it has to be noted that the highest temperature of 28°C of the Bahía Concepción sea surface water temperature corresponds with the cooler temperatures of Puerto Ángel. The temperature is one of the most important external environmental factors that affects molluscan reproduction and in the case of *H. princeps*, for both sexes, the spawning and expulsion stages occurred at relatively low temperatures and the resting period at warmer temperatures of surface waters at the studied locality.

Chlorophyll concentrations have a direct relationship with the development of gonads as this reproductive process demands high energetic quantities that must be obtained from the eaten food extracted from the environment or from reserves previously accumulated or from both [6, 38]. *H. princeps* is a predator

gastropod that depends on the energy obtained from its preys. So, during 2014, for the females, it was noted that when the chlorophyll *a* concentrations were high, maturity and spawning stages presented a higher occurrence percentage and when the concentrations were low, the most frequent gonad stage is resting. For the males, when the chlorophyll concentrations were high, the most frequent gonad stage was the expulsion and when low concentrations occurred, the most frequent gonad stage was resting. In 2015, chlorophyll concentrations were very variable having high values from January to March and an increment in November corresponding with larger percentages of spawning females and expelling males. However, an anomalous high peak of chlorophyll concentration occurred in April when, unlike the same month from 2014, could have caused the reduction of the resting stage and oogenesis and spermatogenesis occurred as indicated by presence of the spawning stage. Therefore, the periods with large chlorophyll availability coincide with the gonad development. *H. princeps* tends to reproduce when the phytoplankton population is blooming, so its offspring could have a higher probability of survival due to food abundance [14, 15].

In relation to temperature is worth to mention that along the period of study, the water temperature differences between surface-bottom lectures were not detected. The direct explanation for this finding is that the rocky coast localities where the individuals of *H. princeps* were collected are places very energetic under the effects of strong wave action, precisely the zones usually inhabited by this organism [7, 8].

In this study, the predominance of reproductive stages occurred during the winter months under relatively colder temperatures. As noted before, the reproduction of the related species *H. erythrostomus* in the Gulf of California happened when the water temperature was around 28°C [37], figure similar to the January surface temperatures of Puerto Ángel.

From October to April but mainly from November to February, the blowing winds, known as the “Tehuano,” originate upwelling and water vertical mixing causing an increase in chlorophyll concentrations [39, 40] by phytoplanktonic blooming. This water mixing process promotes spawning, breeding, and feeding of the aquatic species [41]. Thus, it is possible that the food availability is the main factor affecting the onset of reproduction of *H. princeps* in this region.

On the other side, we would like to mention that the smoothing technique applied to the maturity stage frequencies allow distinguishing in a clearer way the subjacent pattern of the reproductive cycle. With the availability of long-term data records, it is possible to use time series analysis statistical techniques, making it possible to assess the significance of the observed behaviors. From this, it can be stated that *H. princeps* matures and spawns during the cold months of the year (October–March) and rest its reproduction during the warm part of the year (May–August). It observed a positive correlation with the quantity of food (indirectly indicated by the chlorophyll *a* concentrations) and in all the cases, the leads and lags of these variables can be determined [35].

This is the first study on the reproductive cycle of *H. princeps* in the region and present baseline information for: (i) potential management measures, in particular, the knowledge of the timing of spawning season, (ii) assessment of aquaculture potential. More research on the subject is needed.

5. Conclusions

The reproductive cycle of *Hexaplex princeps* was studied through monthly histological analysis of gonads during two annual periods (2014, 2015) from specimens obtained from the artisanal fishery at Puerto Ángel, Oaxaca, Mexico,

considering sexual proportion, gonad maturation, spawning periods, and maturity stages variation in relation with environmental factors (sea surface temperature and chlorophyll concentration).

The sexual proportion was not statistically different from parity, although most of the times, the number of males was slightly larger than the number of females. Only during the spawning season, females were more frequent than males.

The histological analysis permitted to establish maturity stages.

Females (six stages): (1) initial oogenesis, (2) previtellogenic maturity, (3) vitellogenic maturity, (4) maturity, (5) spawning, and (6) resting.

Males (five stages): (1) initial spermatogenesis, (2) maturity, (3) spawning, (4) onset of the rest, and (5) resting.

Monthly variations of maturation stages showed that *H. princeps* has an annual reproductive cycle with a long period of gonadal activity. The spawning season comprised from November to March (females) and from December to March (males) with activity peaks in January. From March to October (females) and May to June (males) reproduction resting occurred.

Reproductive events were related to high chlorophyll *a* concentrations due to the upwelling processes resulting from the predominant winds and to the relatively cooler temperatures proper to the winter (November–March) season at Puerto Ángel, Oaxaca, Mexico.

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Conflict of interest

We state that there is no “conflict of interest.”

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