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Research Article

Reproductive cycle of *Hexaplex princeps* (Broderip, 1833) from one artisanal fishery at the southern coast of Mexico

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ABSTRACT. The reproductive cycle of the gastropod *Hexaplex princeps* (Broderip, 1833) from Puerto Ángel was studied through gonadal histology during two annual periods. The sex proportion for the total 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 histological analysis of gonad sections permitted to establish six maturity stages for females: 1 Initial oogenesis, 2 Previtellogenic maturity, 3 Vitellogenic maturity, 4 Maturity, 5 Spawning, 6 Resting, and five for males: 1 Initial spermatogenesis, 2 Maturity, 3 Spawning, 4 Onset of the rest, 5 Resting). Monthly variations in the gonad 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 peaks of activity for both sexes in January. Resting periods of females comprised from March to October and for males May and June. Thus the reproductive events were related to high Chlorophyll concentrations which in turn are due to the upwelling processes resulting from the winds during winter months (November to March). This study provides baseline information that may serve to establish measures for adequate exploitation and for aquaculture implementation of this species in the future.

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

INTRODUCTION

Marine mollusks constitute one of the more important world fisheries representing around 10% of the total value and quantity (FAO, 2016). These invertebrates have been exploited since ancient times. Moreover, recent studies suggest that the 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 made use of marine shellfish (Marean, 2014). In the world, approximately 720 gastropod species are exploited (Leiva & Castilla, 2002; Elhasni *et al.*, 2013). In Mexico, the gastropod catch in 2013 has the 19th place with 6,011 ton (SAGARPA, 2015).

The gastropod *H. 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 frondlike spines; is gonochoristic and the gonad with the digestive gland occupies the visceral coils (Fretter, 1984); distributes from the Gulf of California to Peru inhabiting moderately shallow waters (Keen, 1971; Morris, 1976). Vega & González (2002) report on the presence of this species in Panama. *Hexaplex princeps* (Broderip, 1833) is captured by several artisanal fisheries of Mexico. According to Landa-Jaime *et al.* (2013) at the Tenacatita

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(Jalisco) coral reef, by its size and taste *H. princeps* is observed with less frequency in the subtidal zone although can be found in the reef searching other gastropods as preys. At Acapulco, *H. princeps* is the second major exploited species after *Striostrea prismatica* (Castro-Mondragón *et al.*, 2015). Our observations along the study period suggest that at Puerto Ángel, Oaxaca this species supports around 80 percent of the gastropod catch in the locality and has fishery importance as is consumed by the local tourism and population. Due to the growing demand and increasing value of appreciated species, the artisanal fisheries are continuously expanding and thus, augmenting the artisanal fisheries effort in the world (Defeo & Castilla, 2005). 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 (Elhasni *et al.*, 2013).

The study of the reproductive processes in marine organisms is a fundamental biological aspect which permits to understand their population dynamics (Underwood & Keough, 2001). The reproductive season is a crucial life-history trait; the proper timing of breeding may be important to provide the offspring with favorable environmental conditions and to influence parental fitness (Varpe *et al.*, 2007; Avaca *et al.*, 2015). 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 members of the Muricidae family (at which *H. princeps* belongs) has been studied in several instances: the gonad cycle of *Bolinus brandaris* at the South of Portugal (Vasconcelos *et al.*, 2012) and at the South of Tunez (Elhasni *et al.*, 2013). In the Gulf of California, Mexico, Cudney-Bueno *et al.* (2008) carried out observations on the oviposition of the “Black murex” *H. nigritus* whose findings were similar to those made before by Wolfson (1968) for *H. erythrostomus*. 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 towards its sustainable exploitation.

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

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, Fig. 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 available 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 to 15 individuals in the interval from 8 to 12 cm in length, (interval that contained more than 90% of the lengths we collected since 2012) were separated and their shell broken to extract the soft parts which were fixed in formalin 10% prepared with seawater according to Ortiz-Ordóñez *et al.* (2009). 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 a pennis.

The sexual proportion was analyzed by means of the chi-squared goodness of fit test following the corrected Yates expression (Sokal & Rolf, 1995; Crawley, 2002).

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 paraplax 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 Hematoxyline-Eosine method (Uría-Galicia & Mora-Vázquez, 1996) 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 surface temperature and chlorophyll of Puerto Ángel data were consulted from the GES DISC-NASA (2016a: 2016b) database. The monthly values were taken from a site in the vicinities of the Puerto Ángel

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

Site	Latitude (N)	Longitude (W)
Punta Cometa (PC)	15°39’35.4”	96°33’16.5”
San Agustinillo (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”

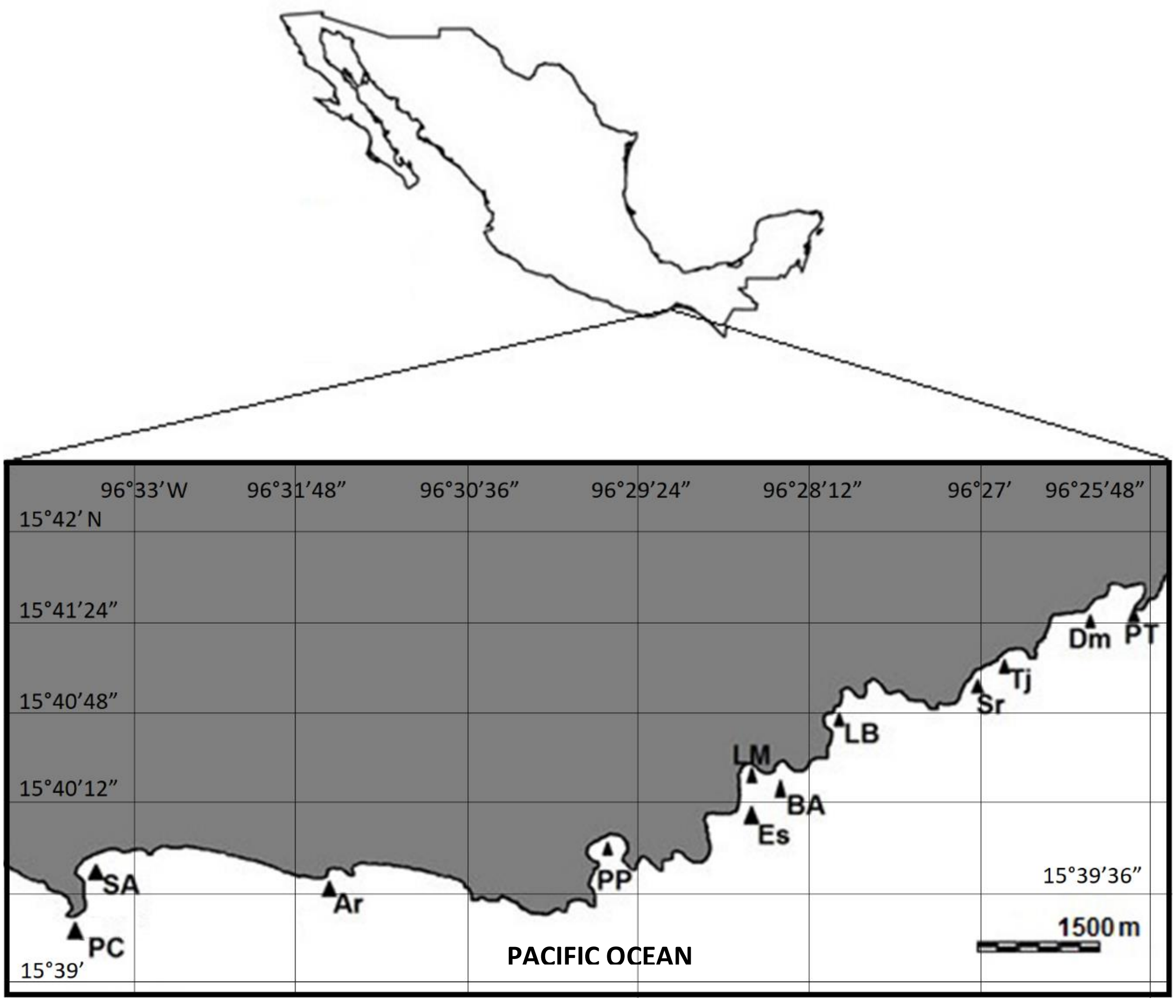

Figure 1. Geographical location of the study area, Puerto Ángel, Oaxaca, Mexico. The collection sites are indicated: PC: Punta Cometa, SA: San Agustinillo, Ar: Aragón, PP: Playa Panteón, Es: Estacahuite, LM: La Mina, BA: Bajos de Aceite, LB: La Boquilla, Sr: Secretario, Tj: Tijera, Dm: Dominguillo, PT: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

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 (Velleman, 1980; Velleman & Hoaglin, 1981; Goodall, 1990; Salgado-Ugarte, 1992, 2017; Salgado-Ugarte & Curts-García, 1992, 1993).

The comparison of the resulting time series data was performed by means of the cross-correlation analysis (Davis, 2002; Becketti, 2013) between the percentages of maturity stages against the temperature and chlorophyll values. Additional cross-correlation analyses among maturity stages were performed too.

RESULTS

A total of 446 individuals, 232 males, and 214 females were analyzed (Table 2) resulting in a sexual male:female proportion of 1.08:1.00 which according to the χ^2 (Yates) test was not significantly different from the 1:1 proportion. No one of the partial samples departed from parity (1:1).

Reproductive cycle

From the 446 captured organisms 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 stages of gonad development were classified as follows: For female six stages were established (Table 2, Fig. 2). Stages 1 initial oogenesis, 2 previtellogenic maturity, 3 vitellogenic maturity, 4 maturity, 5 spawning and finally, 6 resting. For the males, five stages were recognized (Table 3, Fig. 3), 1 initial spermatogenesis, 2 maturity, 3 expulsion, 4 onset of rest, and 5 resting.

Spawning (S5) females of *H. princeps* (Fig. 4) presented large percentages during January (60%), March (67%) and November (67%) of 2014. On the other hand, it is noted that a 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%).

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

Chlorophyll-*a* concentration and gonad cycle

In 2014, the highest chlorophyll 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 these same months the larger frequency of individuals in the spawning stages (S5 females, S3 males) (Figs. 4-5) 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) (Figs. 4-5).

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 the other months the stage with larger frequency was spawning (S5 and S3 for females and males, respectively) (Figs. 4-5). 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 values were related to the resting stage of females and males (S6 and S5, respectively) (Figs. 4-5).

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 (Figs. 4-5).

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 (Figs. 4-5). The months with the highest temperatures were May (30.70°C), June (31.14°C) and August (30.83°C) which were related to the larger frequency of the resting stages of females (S6) and males (S5) (Figs. 4-5).

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 fre-

Table 2. Number of individuals by collecting date and site (main); sexual proportion and its statistical significance are included (χ^2 with Yates correction). Site abbreviations according to Table 1.

Collecting data and site (main)	Total	Males	Females	Male:Female proportion	χ^2 (Yates)	*P*
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

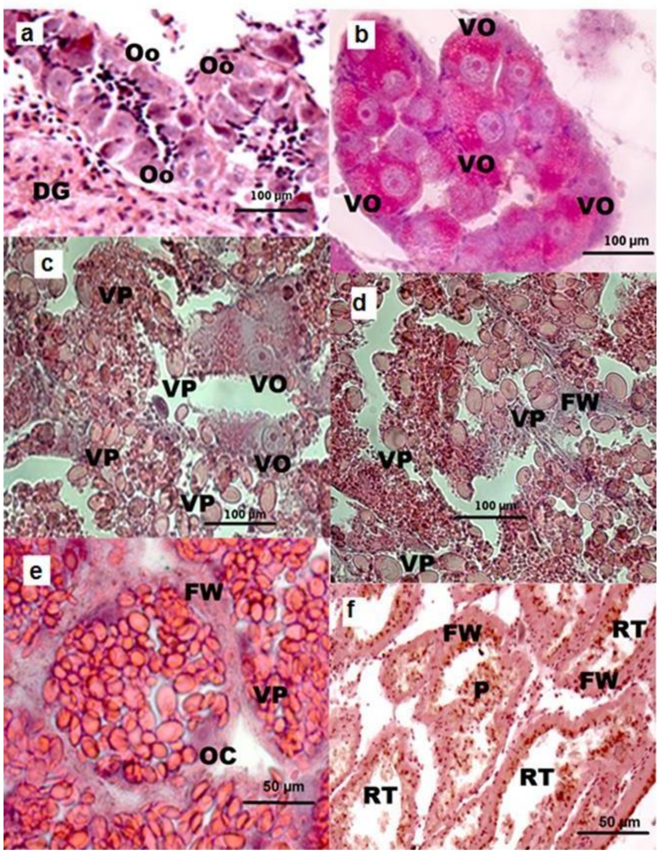

Figure 2. Histological sections of *Hexaplex princeps* females showing ovary stages. a) initial oogenesis (20x), b) previtellogenic mature (10x), c) vitellogenic mature (20x), d) mature (10X), e) spawning (10x), f) resting (10x). Oo: oogonia, Oc: oocytes, DG: digestive gland, CT: connective tissue, PV: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

Table 3. Characterization of gonad development stages for females of *Hexaplex princeps.*

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.

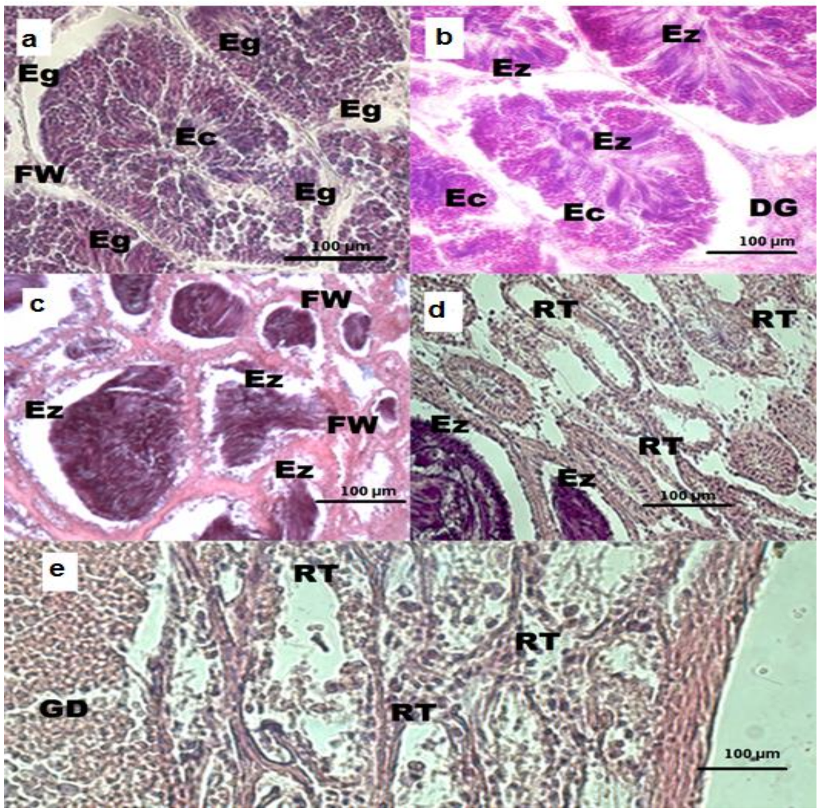

Figure 3. Histological sections of *Hexaplex princeps* males showing testis stages. a) initial spermatogenesis (50 µm), b) mature (100 µm), c) spawning (10x), d) onset of rest (100 µm), e) resting (50 µm). Eg: spermatogonia, Ec: spermatocytes, Ez: spermatozoa, RS: residual spermatozoa, DG: digestive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

quencies of female and male in the spawning stage (S5 and S3, 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 a 35% of stage 2 (maturity), 25% of stage 3 (spawning) and 40% of stage 5 (resting) were observed.

Smoothing and cross-correlation

To describe in more detail the variation and relationships of the reproduction stages and the environmental variables (surface sea temperature and chlorophyll concentrations) along the study period, the smoothed frequency of the reproductive stages were analyzed by cross-correlation. It can be seen easily (Fig. 6) that during the cool months of the year the females mature (S4) from October to February and spawn (S5)

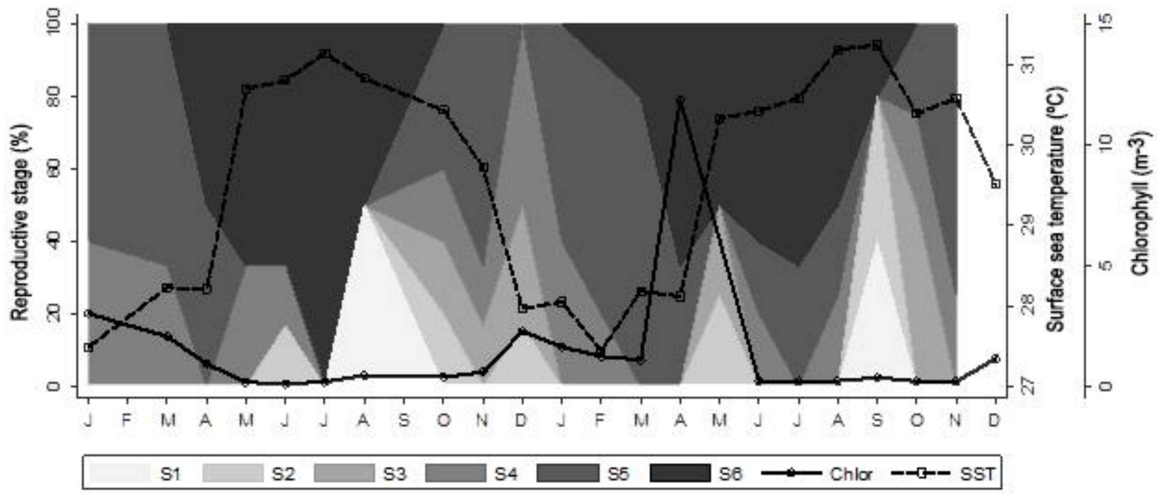

Figure 4. Gonad stage frequency 2014-2015 for *H. princeps* females by sampling date. Chlorophyll concentration (mg m^{-3}) and Surface Water Temperature (°C) values are showed.

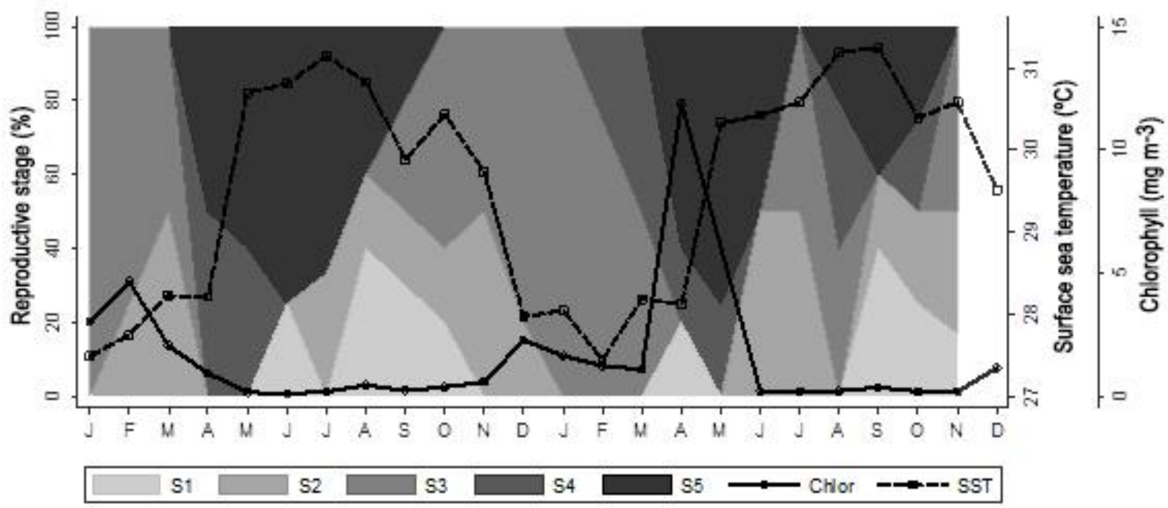

Figure 5. Gonad stage frequency 2014-2015 for *H. princeps* males by sampling date. Chlorophyll concentration (mg m^{-3}) and temperature (°C) values are included.

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 (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 (Fig. 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 more warm months.

The cross-correlograms show a direct relationship lagged 2 months between S2-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 surface sea temperature values with a lag of six months, and concordant (positive) cross-correlation with chlorophyll concentration with no lag (Fig. 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 seven months), and positive with chlorophyll (1-month lag). In contrast with the former

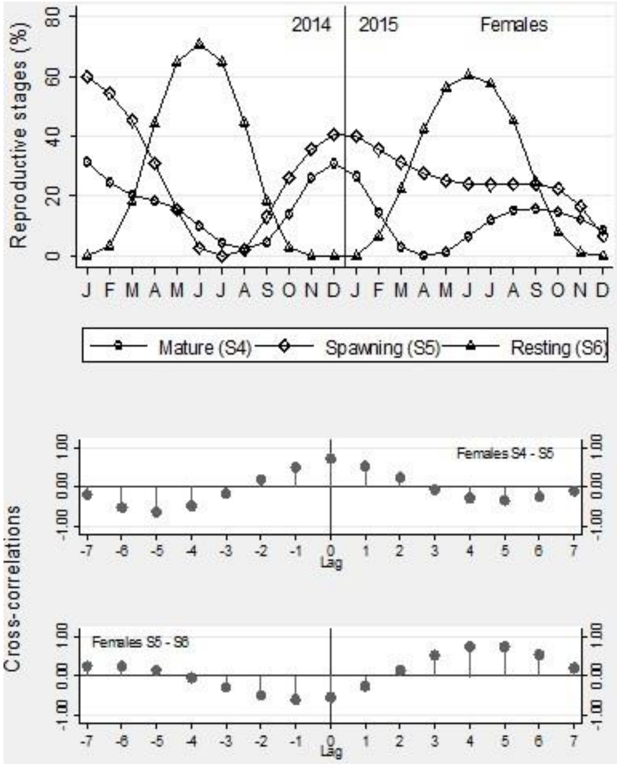

Figure 6. The smoothed frequency of the female reproductive stages along the study period and cross-correlation correlograms for mature-spawning (S4-S5) and spawning-resting (S5-S6) reproductive stages comparison.

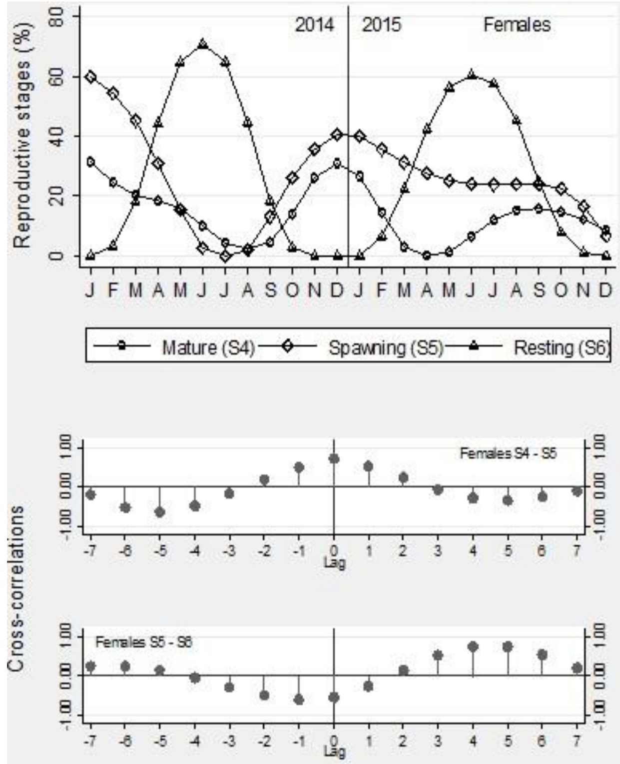

Figure 7. The smoothed frequency of the male reproductive stages along the study period and cross-correlation correlograms for mature-spawning (S2-S3) and spawning-resting (S3-S5) reproductive stages comparison.

trends, the resting females (S6) showed positive cross-correlations (lagged two months) with SST and negative relationship (with a lag of seven months) with the chlorophyll concentrations (Fig. 10). In a similar way as resting females, males in reproductive rest stage (S5) showed a direct trend with SST (lagged one or two months) and the opposite of the chlorophyll values (a lag of seven months) (Fig. 11). A resume of the cross-correlations significances is included in Table 5.

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.* (2008) 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.* (2013) 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), where 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* of 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 stages for *H. princeps*, the stages characterized in the present study for the females correspond closely to those suggested by Vasconcelos, *et al.* (2008) for *H. trunculus* from Portugal. For the 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, the coincident months with spawning and expulsion were January, April, May, October, and November, and the months with the larger frequency of resting individuals were June and July. In

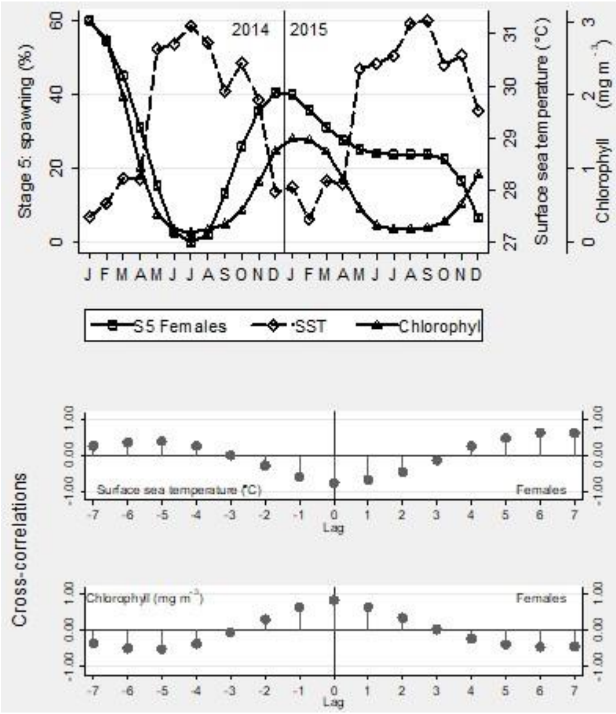

Figure 8. The smoothed frequency of spawning females (S5), surface sea temperature (SST) and chlorophyll concentration (CL) along the study period and cross-correlation correlograms for S5-SST and S5-CL comparison.

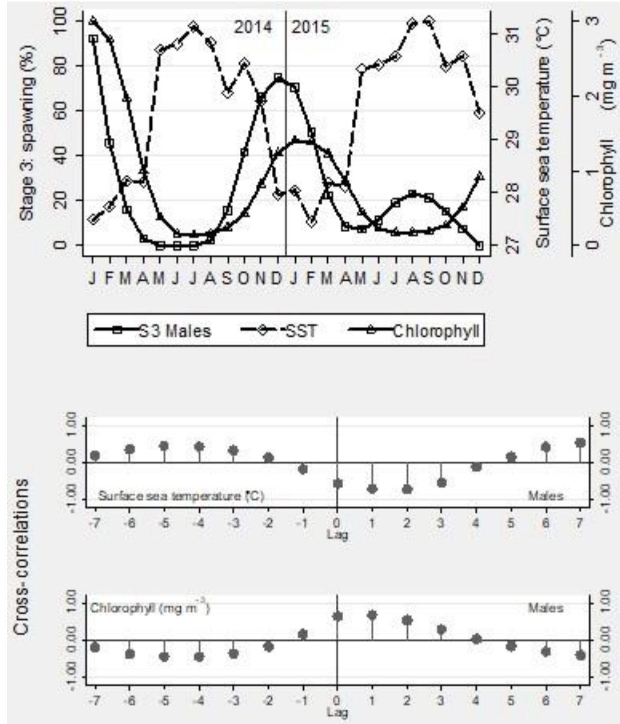

Figure 9. The smoothed frequency of spawning males (S3), surface sea temperature (SST) and chlorophyll concentration (CL) along the study period and cross-correlation correlograms for S3-SST and S3-CL comparison.

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

Stage 1 nitial 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 spermatozoids, sper-matogonia, spermatocytes, and spermatids.
Stage 3 spawning (S3)	Mature spermatozoids 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 spermatozoids are observed; conspicuous conjunctive tissue.

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 not corresponds to the reproduction times reported by Baqueiro-Cárdenas *et al.* (1983) for *H. erythrostomus* from Bahía Concepción, Baja California Sur who establish the reproductive events during the warmer months (May to July), though the highest temperature of 28°C of the Bahía Concepción surface water temperature corresponds with the cooler temperatures of Puerto Ángel. The temperature is one of the external environmental factors most important 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.

Chlorophyll concentrations have a direct relationship with the development of gonads as this 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 (Fretter, 1984; García-Domínguez *et al.*, 2008). *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

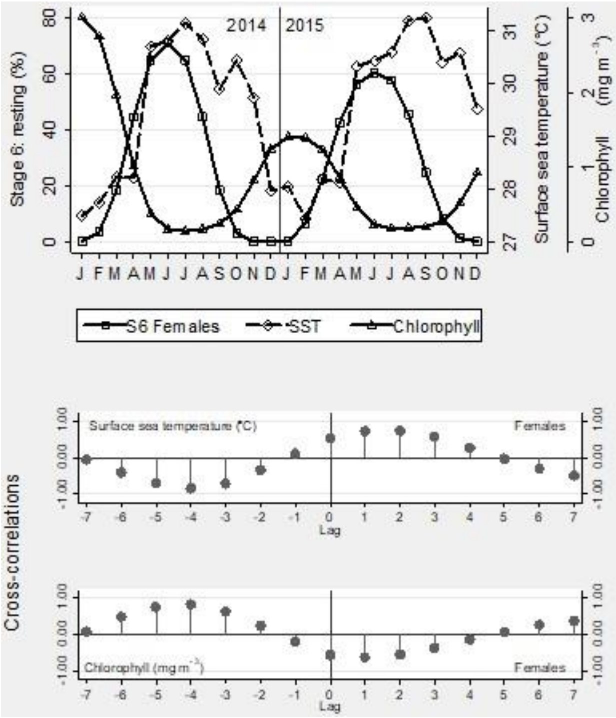

Figure 10. The smoothed frequency of resting females (S6), surface sea temperature (SST) and chlorophyll concentration (CL) along the study period and cross-correlation correlograms for S6-SST and S6-CL comparison.

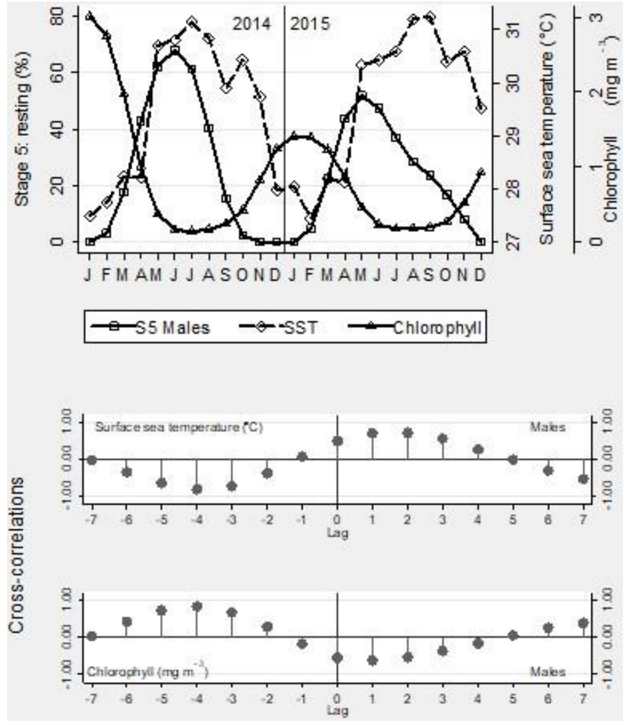

Figure 11. The smoothed frequency of resting males (S5), surface sea temperature (SST) and chlorophyll concentration (CL) along the study period and cross-correlation correlograms for S5-SST and S5-CL comparison.

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

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

concentrations are high, maturity and spawning stages present a higher occurrence percentage and when the concentrations are low the most frequent gonad stages is resting. For the males, when the chlorophyll concentrations were high, the most frequent gonad stages was the expulsion and when low concentrations occurred, the most frequent gonad stages 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 expulsing 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 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 (Varpe *et al.*, 2007; Avaca, *et al.*, 2015).

In relation to temperature is worth to mention that along the period of study there were not detected water temperature differences between surface-bottom lectures. 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 inhabited by this organism (Keen, 1971; Morris, 1976).

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 (Baqueiro-Cárdenas *et al.*, 1983) similar to the January surface temperatures of Puerto Ángel.

From October to April and maxima during the period November to February in the region, the blowing winds, known as the "Tehuanos", originate upwelling and water vertical mixing having higher chlorophyll concentrations (Barton *et al.*, 1993; Trasviña & Barton, 1997). This water mixing process promotes spawning, breeding, and feeding of the aquatic species (Alejo-Plata, 2012). 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 data records, it is possible to use statistical techniques aimed at the analysis of time series, making possible to assess the significance of the observed behaviors. From this, it can be stated that *H. princeps* mature and spawn during the cold months of the year (October to March) and rest its reproduction during the warm part of the year (May to August). It was observed a positive correlation with the quantity of food (indirectly indicated by the chlorophyll concentrations) and in all the cases the leads and lags of the variables can be determined (Becketti, 2013).

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.

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