

Reproductive cycle of the Cortez angelfish *Pomacanthus zonipectus* (Gill, 1863) (Pomacanthidae) from the Gulf of California, Mexico.

Ciclo reproductor del pez ángel del Cortez *Pomacanthus zonipectus* (Gill, 1863) (Pomacanthidae) en el Golfo de California, México.

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ABSTRACT

Different aspects of the reproductive biology of the Cortez angelfish *Pomacanthus zonipectus* were studied between July 1992, and June 1993 at Espíritu Santo Island, Baja California Sur, Mexico. The ovarian and testes development was analyzed using histological techniques. The reproductive cycle was determined and quantitatively analyzed. Five stages of gonadal development were established: resting, developing, ripe, spawning, and spent. *P. zonipectus* is a partial spawner that reproduces from June to November. The reproductive cycle of *P. zonipectus* shows a clear seasonally related with the water temperature, and gonadosomatic index. Gametogenesis started when water temperatures began to increase and continued during warmer months (26°C to 30°C). The interval of size at first sexual maturity was 210-220 mm total length.

Keywords *Pomacanthus zonipectus*, reproduction; gonad; angelfish.

RESUMEN

Se estudiaron diferentes aspectos de la biología reproductora del pez ángel del Cortez *Pomacanthus zonipectus* entre julio 1992 y junio 1993 frente a la isla Espíritu Santo, Baja California Sur, México. Se analizó el desarrollo ovárico y testicular mediante técnicas histológicas. Se determinó y analizó cuantitativamente el ciclo reproductor. Se establecieron cinco fases de desarrollo: reposo, desarrollo, madurez, desove y postdesove. *P. zonipectus* es un desovador parcial que se reproduce de junio a noviembre. El ciclo reproductor de *P. zonipectus* mostró una clara estacionalidad relacionada con la temperatura del agua y el índice gonadosomático. La gametogénesis inició cuando la temperatura del agua empezó a incrementar y continuó durante los meses cálidos (26°C a 30°C). El intervalo de talla de primera madurez sexual fue 210-220 mm longitud total.

Palabras clave *Pomacanthus zonipectus*, reproducción; gónada, pez ángel.

INTRODUCTION

Many marine species have been exploited for exhibition and display purposes. Coral reef fishes have been targeted due to their vivid colors and patterns. The species captured for this trade are known as "ornamental fishes". With the increasing popularity of aquaria in many parts of the world, ornamental fishes have become an important part of international trade (Lem, 2001). However there are few management programs in the marine ornamental trade and those that exist do not have a strong knowledge support. This issue is critical since the exploitations, in some cases, carried on with juveniles. This is the case of the Cortez angelfish *Pomacanthus zonipectus*, which is one of the species with greatest demand by aquarists because of the colour pattern of the juveniles.

In the Gulf of Papagayo, Costa Rica *P. zonipectus* is the third most exploited species in the aquarium trade (Dominici-Arosemena *et al.*, 2001). At present, the collection of *P. zonipectus* is prohibited in the Gulf of California, due to the excessive exploitation level, which has caused the depletion of the population.

In order to meet the future demands of a growing ornamental industry, without depleting the natural population and preserving economic benefits, an alternative is mariculture. At present, only about 30 of more than 800 marine ornamental species which are traded can be reared in captivity (Laidley *et al.*, 2001). One of the major bottlenecks preventing captive production of many marine ornamental species is the reliable control of reproduction in captive brood stock. In this sense, knowledge of the reproductive biology will help in the development of angelfish mariculture.

Studies of the biology of *P. zonipectus* are scarce. Reynolds and Reynolds (1977) reported observations on food habitats in the Gulf of California. Reynolds (1979) reported observations on habitat selection and territorial defence behaviours in juvenile. Moyer *et al.* (1983) reported observations on courtship, spawning, and inferred social organization. Thomson *et al.* (1987) reported from field observations that the breeding of *P. zonipectus* occurs from midsummer to early fall, and that juveniles are most abundant from August through November. Its fecundity was estimated by Arellano-Martínez *et al.* (2006). In general, the reproductive cycle and spawning season of the Pacific species of angelfishes has not been defined using histology, except for *Holacanthus passer Valenciennes* (Arellano-Martínez *et al.*, 1999).

This study is the first report on aspects of the reproductive biology of *P. zonipectus*, including a formal histological analysis of the male, and female gonads and the definition and quantitative analysis of the reproductive cycle and its relationship with water temperature.

MATERIAL AND METHODS

Samples were collected monthly (except in August) with a Hawaiian speargun at 2-10 m depth, between 10:00 and 14:00 hours, at Espiritu Santo Island (24°24'-24°33' N, 110°21'-110°24' W) in the Gulf of California, Mexico. A total of 120 specimens of *P. zonipectus* were collected between July 1992, and June 1993. It was necessary to keep the sample size relatively small because the *P. zonipectus* population in our study area was small, with a relative abundance between 0.01 and 0.1 (calculated as the ratio between the number of individuals of *P. zonipectus* and the total number of fishes in the region) (Pérez-España & Abitia-Cárdenas 1996; Aburto-Oropeza & Balart, 2001). Total length (TL), and total weight (TW) were recorded for each specimen. The water temperature was recorded at the time of sampling.

The gonads of all specimens were removed, weighed and fixed in 10% formalin solution for further histological analysis. Gonad sections were dehydrated in alcohol, and embedded in paraffin. They were cut at 7µm in cross section and stained with haematoxylin-eosin (Humason, 1979).

Sex was determined by histological analysis and the gonads were classified, according to the stages (resting, developing, ripe, spawning and spent) established by Arellano-Martínez *et al.* (1999) for *H. passer*. The relative frequencies of each gonadal development stage throughout the annual cycle were obtained. The spawning season was defined by the presence of ripe and spawning individuals.

The accumulated relative frequencies of mature females by 10 mm length intervals were calculated, and the length category with 50% of mature females was considered as the interval of size at first sexual maturity.

The sex ratio during the study period was obtained monthly and with all data pooled as the number of females/number of males.

The reproductive condition of specimens was also examined with a gonadosomatic index (GSI) calculated as $GSI = \text{gonad weight (g)} / (\text{total weight} - \text{gonad weight (g)}) \times 100$. The monthly GSI means and standard errors were obtained to determine the general trends of reproductive activity. Following Arellano-Martínez *et al.* (1999), a monthly gonad index (GI) was calculated for each sex to make a quantitative comparison of GSI with histological results, utilizing a numerical grading system with 1 = resting and spent, 2 = developing, and 3 = ripe and spawning.

Statistical analysis. Spearman's correlations were applied between mean GSI values and the GI values to verify the usefulness of GSI as an indicator of the reproductive activity of this species. Correlation analyses also were used to determine the relationship between GSI, GI, and temperature. The correlation

analyses were done with all the data from specimens captured during the annual cycle, except the immature females. As GSI is a percentage value, the arcsine transformation (Zar, 1996) was applied, but data are shown as percentages. ANCOVA (by sex) with body weight as the covariable were also applied to assess significant differences in GSI between months (with this, the effect of individual body weight was eliminated). Duncan's multiple range post-hoc mean comparison tests were performed to determine the origin of the differences. The sex ratios were analyzed with a χ^2 test for significant deviations from the expected ratio (1:1).

STATISTICA software (version 5.0) was used for all statistical analyses. The level of significance (α) was set at 0.05 for all analyses.

RESULTS

Sex ratio. A total of 120 specimens were collected, 54 were females (45 %) and 66 were males (55.0 %). The sex ratio by month (Table 1) shows variability, but no significant differences from the expected ratio (1:1) were found ($P > 0.05$). The total sex ratio (0.8 females : 1 male) was not significantly different ($P > 0.05$) from the expected ratio (1:1). Fishes ranged in TL from 150 mm to 349 mm (female range 150-262 mm TL, male 182-349 mm TL). The mean size of all specimens pooled was 237.7-mm TL (± 38.0 -mm SD).

Gonadal stages. The Cortez angelfish has cellular types similar to most teleosts, described by West (1990), with the (simultaneous) presence of oocytes at different stages of development. The general pattern of gonadal development of *P. zoni-*

pectus included resting, developing, ripe, spawning, and spent stages. Additionally, an immature ovary stage was observed.

Resting. Ovary: Oocytes were embedded in finger-like folds, the predominant oocytes were early and previtellogenic oocytes. Gonadal lumen was generally large (Fig. 1a). Testicle: Little or no spermatocyte development present. Spermatogenic tubules, in general, were inactive (Fig. 2a).

Developing. Ovary: Large early and previtellogenic oocytes were in lesser quantities. The vitellogenic and mature oocytes were dominant. The lumen was smaller than at the previous stage (Fig. 1b). Testicle: Intense spermatogenic activity in the tubules with cells in all stages of development. Spermatozoa collecting in spermatogenic tubules (Fig. 2b).

Ripe. Ovary: The mature oocytes were the more representative ones. Prehydrated and hydrated oocytes were present in some ovaries. There was no lumen visible (Fig. 1c). Testicle: The spermatozoa was predominant with little or none spermatogenic activity occurring. The collecting tubules were totally filled with spermatozoa (Fig. 2c).

Spawning. Ovary: Not observed. Testicle: Channelling of spermatozoa into collecting tubules. The spermatogenic and collecting tubules were partially empty (Fig. 2d).

Spent. The abundance of early oocytes increased. The vitellogenic and mature oocytes were in reabsorption. The atretic structures were dominant, so that the gonad could be considered in reabsorption (Fig. 1d). Testicle: Tubules almost empty, with some residual spermatozoa. Some spermatogenesis may occur (Fig. 2e).

Table 1. Female and male frequencies, sex ratios and Chi square (χ^2) values of *Pomacanthus zonipectus* by month. There were no significant differences ($P > 0.05$) in sex ratio from 1:1.

Month	Females	Males	Total (N)	Sex ratio F:M	χ^2
July 1992	10	7	17	1.42:1	0.03
August		NO DATA			
September	4	8	12	0.50:1	0.11
October	6	14	20	0.43:1	0.16
November	4	1	5	4.0:1	0.36
December	3	2	5	1.5:1	0.04
January 1993	3	6	9	0.5:1	0.11
February	8	8	16	1:1	0
March	4	5	9	0.8:1	0.01
April	3	5	8	0.6:1	0.06
May	4	7	11	0.57:1	0.07
June	5	3	8	1.66:1	0.06
Total	54	66	120	0.82:1	0.01

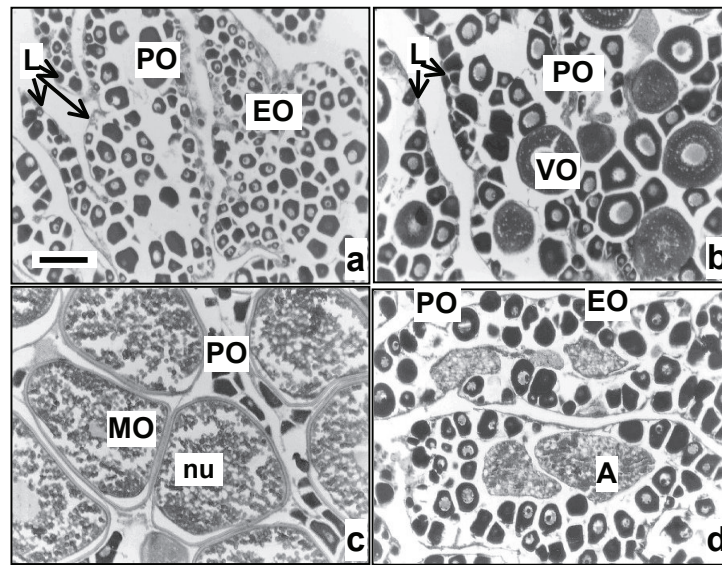


Figure 1. Photomicrographs of stages of ovary development. a) resting ovary, b) developing ovary, c) ripe ovary, d) spent ovary. Hematoxylin-eosin stain. Abbreviations: A, atresia; EO, early oocytes; L, ovarian lamella; MO, mature oocyte; nu, nucleus; PO, previtellogenic oocytes; VO, vitellogenic oocyte. Scale bar = 150 μ m.

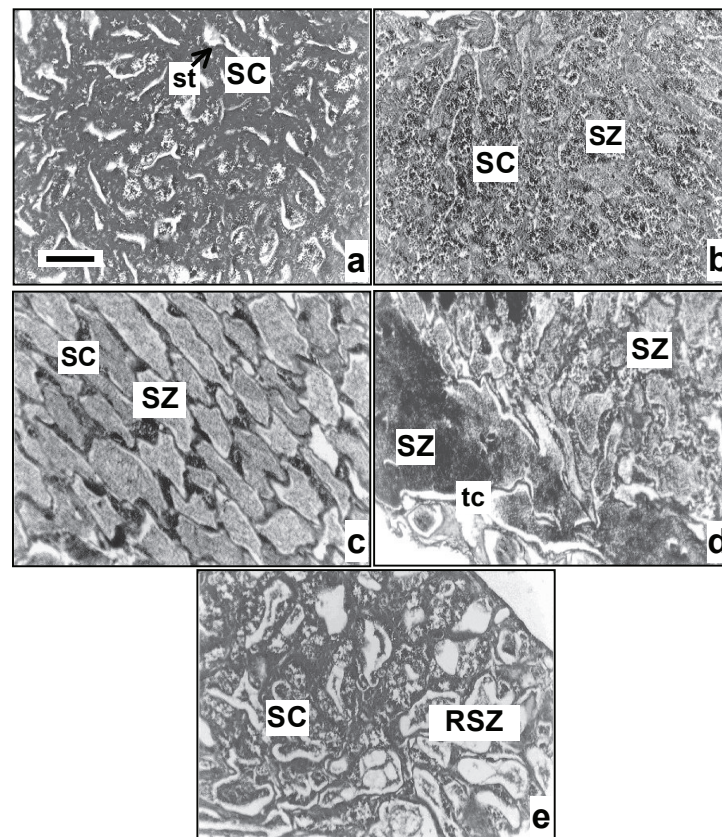


Figure 2. Photomicrographs of stages of testis development. a) resting testis, b) developing testis, c) ripe testis, d) spawning testis, e) spent testis. Hematoxylin-eosin stain. Abbreviations: RSZ, residual spermatozoa; SC, spermatocytes; st, spermatogenic tubule; SZ, spermatozoa; tc, collecting tubule. Scale bar = 150 μ m.

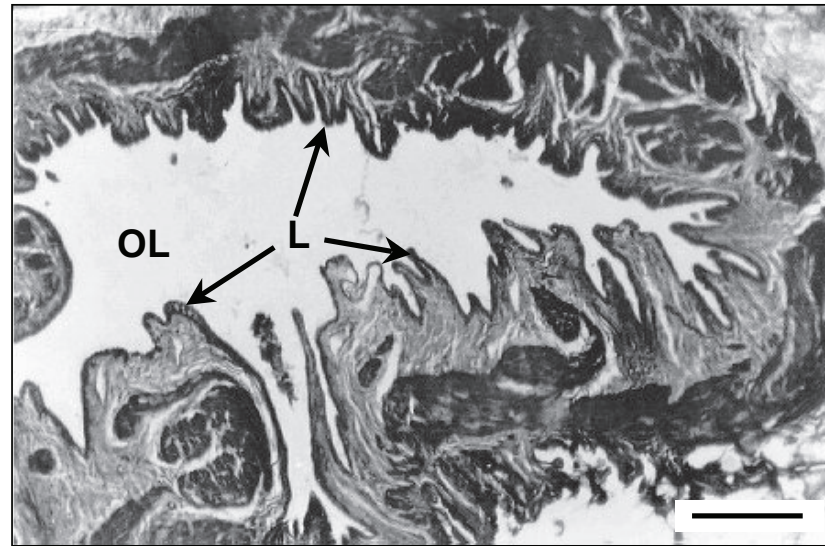


Figure 3. Photomicrograph showing an immature ovary of *Pomacanthus zonipectus*. Hematoxylin-eosin stain. Abbreviations: L, ovarian lamella; OL, ovarian lumen. Scale bar = 150 μ m.

Immature ovary. A large ovarian lumen was evident, with developing ovarian lamellae but without evidence of gametogenic development (Fig. 3).

Reproductive cycle. The reproductive cycle of *P. zonipectus* is shown in figure 4. Immature females were not included in the reproductive cycle analysis. Both sexes have the same tendency with a resting period from November to March determined by the dominance of the resting and spent stages. The gonadal development started in March for males and in April for females. Despite females with features of spawning (post-ovulatory follicles) were not found, the ripe females occurred from June to November while ripe and spawning males occurred from May to November suggesting that the spawning season of *P. zonipectus* comprises these period.

Table 2. Frequencies of mature *Pomacanthus zonipectus* females by interval of size. Interval of size at first sexual maturity in bold font.

Length interval	Frequency	Relative frequency (RF)	Accumulated RF
160-160	1	3.7	3.7
160-170	0	0	3.7
170-180	0	0	3.7
180-190	2	7.4	11.1
190-200	4	14.8	25.9
200-210	4	14.8	40.7
210-220	3	11.1	51.8
220-230	4	14.8	66.7
230-240	6	22.2	88.9
240-250	1	3.7	92.6
250-260	2	7.4	100

Quantitative analysis of reproductive activity. Monthly GI and GSI values are shown in Figure 5a and 5b. The GI of female and male had a similar tendency all year round with a significant positive correlation ($R = 0.75$; $P < 0.01$). High values coincided with reproductive activity (June to November). The GSI values were low from October to May for females ($< 1.03\%$) and from November to May for males ($< 0.08\%$). In June, the GSI reached the highest value for both sexes (female, 8%; male, 0.14%). Whereas, from October/November to May the GSI values were low. A significant correlation for females ($R = 0.92$; $P < 0.001$) was found between GI and GSI.

Applying ANCOVA with body weight as the covariable and computed independently for each sex, differences of GSI between months for both, females ($F_{10,40} = 11.3$; $P < 0.001$) and males ($F_{10,54} = 4.4$; $P < 0.001$) were detected. With the Duncan's test three groups for females and two groups for males, were detected. In females, the first group included June (showing the highest GSI mean value 8%), the second group included July, and September (showing GSI mean values of 2.8 and 2.7%, respectively), and finally the third group included from October to May (showing the lower GSI mean values, $< 1.03\%$). In males, the first group included June, July, September, and October (showing the higher GSI mean values $> 0.1\%$). The second group includes from November to May, showing low GSI mean values ($< 0.08\%$).

Relation of water temperature and reproductive cycle. Monthly changes in water temperature were found, from 20.6°C (February) to 30.1°C (October) (Fig. 5c). The higher values were from June to November (coinciding with reproductive activity), whereas the lower values were from December to February.

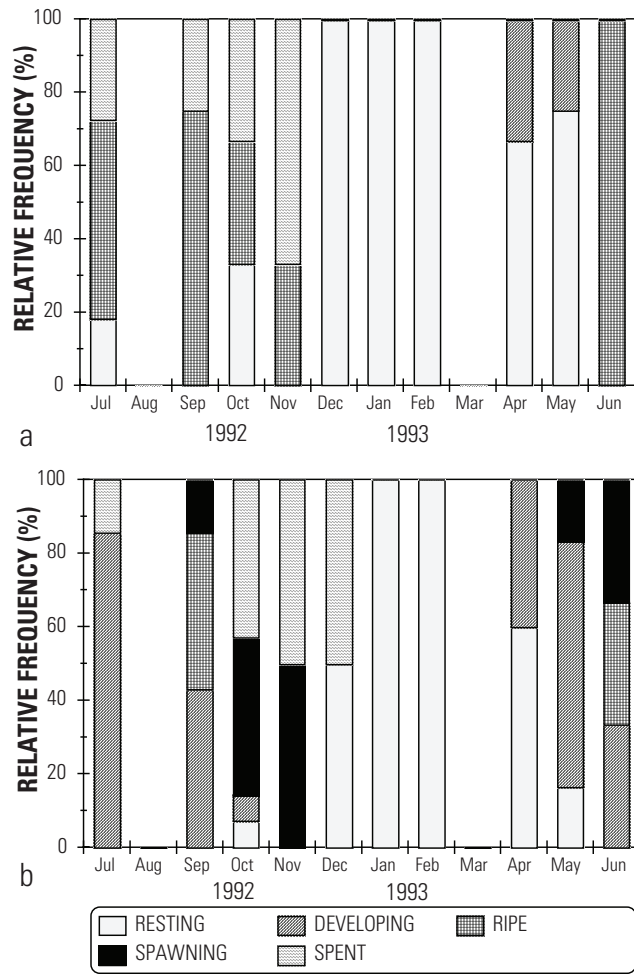


Figure 4. Reproductive cycle of a) females and b) males of *Pomacanthus zonipectus* between July 1992 and June 1993. In August no sampling was made and in March the gonads were lost.

For both sexes, GSI and GI were positively correlated with the water temperature, however only in females significant correlations between the water temperature, and GSI ($R = 0.645$ $P < 0.05$) or GI ($R = 0.75$ $P < 0.01$) were found.

Interval of size at first sexual maturity. The length at first sexual maturity of *P. zonipectus* females was observed in the interval of size 210-220 mm TL (Table 2).

DISCUSSION

The characteristics of gametogenesis in the Cortez angelfish *P. zonipectus* were similar to those described for the king angelfish *H. passer* (Arellano-Martínez et al., 1999). The ovaries of *P. zonipectus* have batches of oocytes at different stages of development simultaneously, a characteristic common in fishes with group synchronous ovaries (Wallace & Selman, 1981; West, 1990; Salgado-Ugarte et

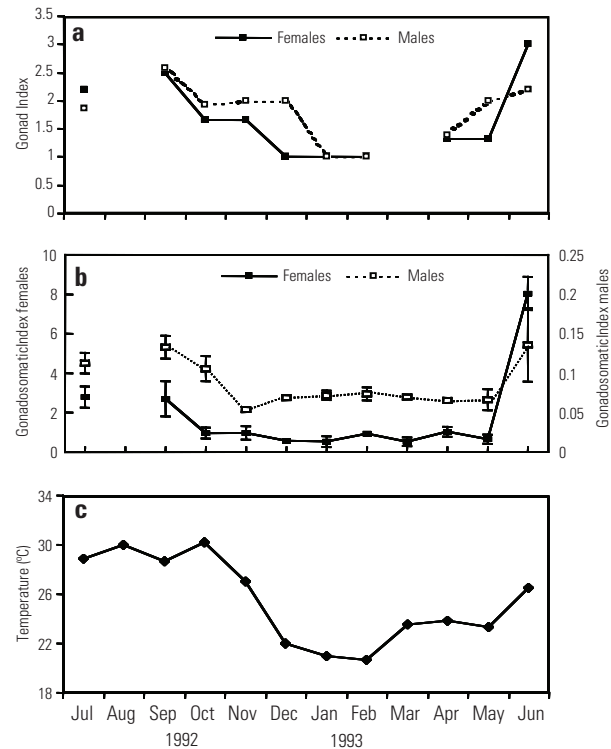


Figure 5. Monthly variations of a) gonad index, b) gonadosomatic index for *Pomacanthus zonipectus*, and c) water temperature at Espiritu Santo Island, B. C. S., Mexico from July 1992 to June 1993.

al., 2005) and partial or multiple spawning (García-Cagide & García, 1996; Ceballos-Vázquez & Elorduy-Garay, 1998). Also, atresia that is indicative of previous spawning was present in ovaries with oocytes in a clear developing stage. The above suggests that these species had more than one spawning during the reproductive period such as in other reef fishes (Arellano-Martínez et al., 1999; Ruiz et al., 1999).

The lack of females in spawning condition (presence of postovulatory follicles) could be due to the assumption that the spawning may have occurred at twilight, such as has been documented for other pomacanthids (Moyer & Nakazono, 1978). Considering the sampling time (10:00 to 14:00 hours), it is probably that the postovulatory follicles had been reabsorbed or in an advanced stage of reabsorption, and that in the histological analysis they corresponded to late atresia.

Few information on the reproductive biology of the angelfishes exist, but invariably it suggests a strong seasonal change in their reproductive activity (Thresher, 1984). In this sense, it is well established that water temperature is one of the most important environmental factors in the physiological regulation of fish reproduction (Moyle & Cech, 1988), and the main factor responsible for reproductive seasonality, spawning trigger, and the rate of gonadal development in fishes (Bye, 1990). In some fishes, sudden increases in water temperature appear to be the

final cue for stimulating maturation and ovulation (Bye, 1990), and cooler temperatures may inhibit gametogenesis. For other species of fish like *Mugil cephalus*, *Dicentrarchus labrax*, and *Sparus aurata*, this relationship may be the inverse of that stated above (Zohar, 1989).

In temperate regions, the angelfishes spawn during the warmer months (Thresher, 1984). Thomson *et al.* (1987), reported that breeding of *P. zonipectus* occurs in the Gulf of California from midsummer to early fall. Similarly, in this study the reproductive cycle of *P. zonipectus* showed a clear seasonality related to the water temperature, which was evident by the significant positive correlation found between water temperature and female GSI. The relationship of reproductive activity with water temperature in pomacanthids had been observed in other studies (Thresher, 1984; Moyle & Cech, 1988; Arellano-Martínez, *et al.*, 1999).

The maximum GSI values for *P. zonipectus* were detected from summer to fall (when the gonads were ripe) with water temperatures from 26°C to 30°C. Whilst the lowest GSI values were observed in winter (when the gonads were resting) with water temperatures from 20°C to 22°C. Spawning males occurred when water temperature was found between 23-30°C. Thus, gametogenesis started when water temperatures began to increase and continued during the warmer months (26°C to 30°C).

Although, the GSI does not give exact information on gonadal development stage; it is useful to help define the seasonal trend in the reproductive activity of a population and, provides estimations of the period of spawning (De Vlaming *et al.*, 1982; Erickson *et al.*, 1985; Cayre & Laloé, 1986; West, 1990). The Cortez angelfish had a seasonal trend in the GSI mean monthly values of females that generally agrees with the reproductive activity and with histological results. The above was reinforced with the significant positive correlation obtained between female values of GSI, and GI. Therefore, the monthly trends in GSI are predictive in comparison to reproductive condition, then the GSI adequately represents the main period of reproductive activity of female *P. zonipectus* (June to November). The same was reported for other fishes (Ceballos-Vázquez & Elorduy-Garay, 1998; Arellano-Martínez *et al.*, 1999; Ruiz *et al.*, 1999). On the contrary, in males the correlation between GSI and GI was not significant, therefore GSI do not represent adequately the reproductive activity of males. This result may be explained for the low weight that the male gonads reach during the maturation process.

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