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Research Article

Reproductive biology of the leopard grouper *Mycteroperca rosacea* (Streets, 1877) in the coastal area of Santa Rosalía, BCS, Mexico

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ABSTRACT. The leopard grouper *Mycteroperca rosacea* is endemic to northwestern Mexico. It has been classified as vulnerable by the IUCN since 2008. *M. rosacea* has high commercial value and is caught year-round in Baja California Sur (BCS). Biological information on this species, especially in its natural environment, is scarce, and the objective of this study was to analyze its reproductive biology in Santa Rosalía, BCS. A total of 345 specimens were collected from March 2014 to May 2015. The sex ratio was 2.0: 1.0 (females: males, $P < 0.05$). Population size at first maturity (L_{50}) was estimated at 40.77 cm TL; 37.31 cm TL for males and 42.44 cm TL for females. Gonadic development was synchronous by group, with a reproductive period occurring from March to May in 2014 and 2015 (at 20-23ºC). The gonadosomatic index achieved maximum values in May 2014 and March 2015, as did the highest frequencies of mature and spawning individuals, indicating that there was a clear seasonal pattern of reproduction, and a negative correlation with temperature for both sexes. The hepatosomatic index and the condition index indicated that *M. rosacea* is not a species that requires storing of energy for reproductive events, as it seems to have food available year-round.

Keywords: *Mycteroperca rosacea*, size at first maturity, reproductive cycle, somatic indexes, temperature.

INTRODUCTION

The leopard grouper *Mycteroperca rosacea* (Streets, 1877) is distributed from the southwestern coast of the Baja California Peninsula in the Gulf of California to Jalisco (Heemstra & Randall, 1993), and is therefore considered endemic to the Mexican Pacific. Due to its restricted distribution, as well as to its reproductive biology, it is cataloged as vulnerable and has been included in the Red List of threatened species by the International Union for the Conservation of Nature (Craig & Sadovy, 2008). This species is considered an iteroparous species, cataloged until now as having gonochoric species (Erisman *et al*., 2008); it forms reproductive aggregations from March to June, depending on location (Aburto-Oropeza *et al.*, 2008). In addition to being a predictable event, the increase in the abundance of individuals at reproductive sites results in the species being vulnerable to overexploitation, as capture efforts usually are focused on those sites. It is a relatively easy way to obtain a large catch volume, and the impact of extraction on reproducing individuals can cause more significant long-term effects on the population, as it reduces significantly the number of individuals that participate in the reproductive event (Russell, 2001).

The leopard grouper is considered a high-quality species (Aburto-Oropeza *et al.*, 2008); it is the third fish species regarding catch volume in Baja California Sur (BCS), and BCS has the highest catch volume in the country. The highest recorded catches in BCS were of 670 ton (CONAPESCA, 2013). Despite the economic importance of this species, no management plan has been developed, and there are no established catch quotas or daily fishing limits for its commercial fishery within the Gulf of California (Erisman *et al.*, 2007). The importance of the leopard grouper is not restricted only to the economic environment; it is also important

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ecologically because species belonging to the Serranids are considered active predators and are located at the upper levels of the food chain (Peláez-Mendoza, 1997).

This species, especially the wild populations within the Gulf of California, have been little studied. We focused on the reproductive biology of *M. rosacea* in the coastal area of Santa Rosalía, BCS, to determine its reproductive cycle, especially its duration and intensity, compared with other areas of the Gulf of California, and establish the possible effect of water temperature on its reproduction.

MATERIALS AND METHODS

Sampling was carried out monthly from March 2014 to May 2015 along the coastal zone of Santa Rosalía, BCS. This location belongs to the municipality of Mulegé, in the central area of the eastern coast of the Baja California Peninsula (Fig. 1).

Fish were caught by artisanal fishery methods using barbed fishing spears. The total length (TL), total weight (TW), gonad and liver weight were recorded for each organism. Gonads were preserved in Davidson AFA solution (glycerin, formaldehyde, ethylic alcohol, filtered seawater, and acetic acid) for later histologic processing and sex determination (Estrada-Godínez *et al*., 2011). The sex ratio was calculated by dividing the number of individuals of each sex. A 1:1 sex ratio was hypothesized; the χ^2 statistical test with Yates correction was used to corroborate this, with $\alpha = 0.05$ for all tests (Sokal & Rolf, 1995).

Gonads were processed using the standard histological technique to determine cellular types, annual variability, and gonad developmental stages. Gonads were dehydrated at increasing ethanol concentrations and embedded in paraffin. 4 µm thick cuts were obtained using a microtome (Leica RM 2155). Tissues were mounted and stained with hematoxylin-eosin, and additional stains were performed using Mallory´s trichrome stain procedure.

Five developmental stages were considered for females, taking into account the oocyte development description by Estrada-Godínez *et al.* (2011). a) Primary growth, consisting of three stages (chromatin nucleus, early perinucleolus, and late perinucleolus); b) Secondary growth: oocytes having cortical alveoli and lipid inclusions; c) Vitellogenesis: oocytes presenting primary and secondary yolk granules; d) Final maturation: characterized by hydrated oocytes with migrated nucleolus and post-ovulatory follicles, and e) Spawned: during this stage the unspawned oocytes are reabsorbed (atresia). Three stages were identified in males. a) Regressed: in which a considerable quantity of spermatogonia were observed dominating almost the entire testicle, although some areas with spermatozoid presence were also found, b) mature: during which spermatocytes were observed over almost the entire testicular tissue, with high number of spermatozoids, c) spent: where practically the entire testicular tissue presented spermatozoids, with spermatic sacs partially full. There was an additional stage in males called d) reabsorption: during which a considerable quantity of conjunctive tissue without apparent organization, but with a few spermatozoids, was observed.

The following somatic indexes (Shulman & Love, 1999) were used to evaluate the physiological condition of individuals:

Gonadosomatic index: $GSI = \frac{GW}{TW} \times 100$

Hepatosomatic index: $HSI = \frac{LW}{TW} \times 100$

Condition factor: $CF = \frac{TW}{TL^3} \times 100$

where: GW = gonad weight; TW = total weight (eviscerated); LW = liver weight; TL = total length.

The data obtained with the somatic indexes (GIS, HSI, and CF) were expressed as percentages and arcsine square root transformed. A one-way analysis of variance (ANOVA) was used to compare the averages of these indexes. Duncan´s test was then used to determine significant differences ($P > 0.05$) between sampling months and somatic indexes (Estrada-Godínez *et al.*, 2011).

The reproductive season was delimited according to different stages of gonadic development and to the variations presented in the somatic indexes, following Estrada-Godínez *et al*. (2011); 1) maturation: observed from the beginning of vitellogenesis in oocytes until the final maturation stage; males can be observed in a maturity state; 2) spawning: the oocytes are spawned in the water and fertilized by males; 3) post-spawning: the females have postovulatory follicles, and the atresia process can be observed, whereas most males are spent; and 4) resting: only oogonia and oocytes undergoing primary growth can be observed in females, whereas males are in a regression state.

The size at first maturity $L_{50\%}$ of the species and for each sex was calculated with the percentage of spawning (females), and ejaculated (males) in each length range (6.3 cm in length) compared to the total number of individuals sampled of this stages. The logistic distribution of percentage of mature and spawned fish grouped at intervals and $L_{50\%}$ were obtained accord to criterions of Taylor & Murphy (1992). Statistica v.8 program was used to obtain the logistic distribution variables.

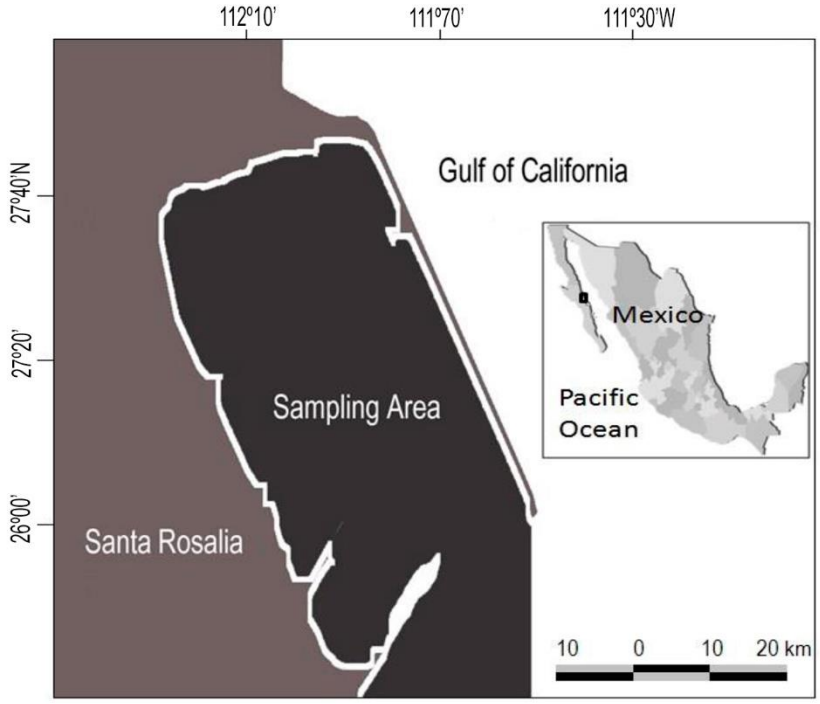

Figure 1. Map showing the sampling area of the leopard grouper *Mycteroperca rosacea* in Santa Rosalía, Mexico.

Average sea surface temperature images, extracted from composite images acquired from Aqua-MODIS and Terra-MODIS sensors, were obtained from the Scripps Institution of Oceanography. These images were of High-Resolution Picture Transmission (HRTP) in Hierarchical Data Format (HD). Images were processed using the Windows Image Manager© (WIMSoft) program to obtain sea surface temperatures. A Spearman´s test was carried out to identify whether there was a correlation between temperature and somatic indexes (Statistica v.8).

RESULTS

Three hundred and forty five *Mycteroperca rosacea* specimens were obtained. Total length (TL) ranged from 21 to 74 cm. Ninety-three individuals were males (26.7%), 185 were females (53.6%), 15 were bisexual immature (4.6%), and 52 could not be identified (15.1%). Females ranged in size from 21 to 74 cm TL, males measured from 24 to 68 cm TL, immature bisexual measured from 30 to 68.5 cm TL, and unidentified individuals measured from 24 to 49 cm TL (Fig. 2).

The sex ratio for the entire sample was 2.0:1.0 (females: males), with significant differences (χ^2 = 48.41, d.f. = 1, $P < 0.05$) from the expected 1:1 proportion. Females were significantly more abundant in October and November 2014 (Table 1). Immature bisexual individuals were recorded at a low percentage during almost the entire year of sampling (Fig. 3). The spawning period occurred from March to May in 2014 and 2015, at temperatures of between 20.3 and 22.2ºC (Fig. 4).

Immature bisexual individuals were identified following criteria by Estrada-Godínez *et al.* (2011). These individuals presented primary growth oocytes as well as spermatogonia, spermatocytes, and spermatozoids (Figs. 5a-5b).

Presence of spermatocytes was observed in males, as well as spermatozoids in the spermatic lobes and spermatic sacs (Fig. 5c). Females with gonads containing cells at the primary growth stage were observed, but there were also females beginning the maturity process, with secondary growth oocytes, vitellogenesis, and spermatocysts (Fig. 5d).

Significant differences ($P < 0.05$) in the GSI were observed between months for males as well as for females, but there were no significant differences between the sexes ($P > 0.05$). Maximum male and female GSI values occurred from March to May in 2014 and 2015, coinciding with the spawning seasons (Fig. 6).

There were significant differences in HSI between months when males and females were analyzed separately ($P < 0.05$), but there were no significant

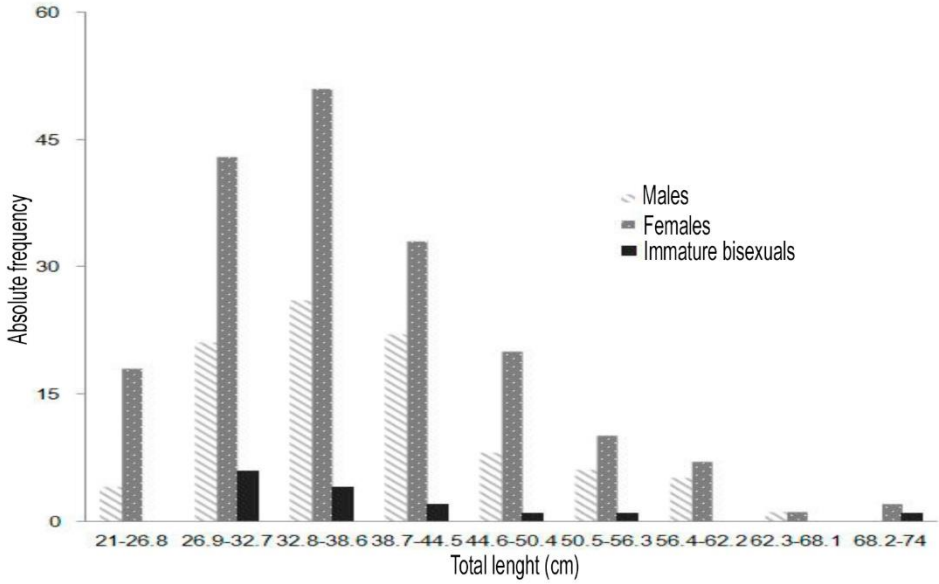

Figure 2. Size-Frequency distributions of males (n = 93), females (n = 185) and immature bisexuals (n = 15) of *M. rosacea*.

Table 1. Monthly sexual ratio and χ^2 values with Yates correction factor for *M. rosacea*. *Indicates significant differences ($P < 0.05$).

Month	Females	Males	Total	χ^2	P	Ratio (F:M)
March	14	6	20	3.2	0.1	2.3:1.0
April	21	11	32	3.13	0.1	1.9:1.0
May	20	19	39	0.03	0.5	1.1:1.0
July	3	1	4	1.0	0.35	3.0:1.0
August	6	3	9	1.0	0.35	2.0:1.0
September	8	2	10	3.6	0.1	4.0:1.0
October	11	5	16	2.25	0.15	2.2:1.0
November	21	4	25	11.56*	0.001	5.3:1.0
December	16	1	17	13.24*	0.001	16.0:1.0
January	6	8	14	0.29	0.5	0.8:1.0
February	5	5	10	0	0.5	1.0:1.0
March	23	12	35	3.46	0.1	1.9:1.0
April	14	5	19	4.26*	0.05	2.8:1.0
May	17	11	28	1.29	0.3	1.5:1.0
Total	185	93	277	48.31	-	2.0:1.0

differences between sexes ($P > 0.05$). There was no relationship between the maximum or minimum HSI values and GSI for either sex (Fig. 6).

There were significant differences in CF between months for each sex ($P < 0.05$) but there were no significant differences between males and females ($P > 0.05$). Maximum CF values were not related to the GSI of either males or females (Fig. 6).

Individual size at first maturity of *M. rosacea* females was 23.9 cm TL, that of males was 24 cm TL, and the minimum size of immature bisexual individuals was 26 cm TL (Fig. 7).

The *M. rosacea* L_{50} for the coastal area of Santa Rosalía, BCS, estimated by adjusting a logistic curve, was 40.8 cm TL for both sexes combined, 37.3 cm TL for males, and 42.4 cm TL for females (Fig. 8).

The maximum superficial sea temperature in 2014 was 30ºC in July, and the minimum was 18ºC in January. When there was an increase in temperature linked with the warm season, gonads started their gametogenic development, which continued until maturity and spawning; when the temperature reached 30ºC, however, the population entered a resting stage (Fig. 9).

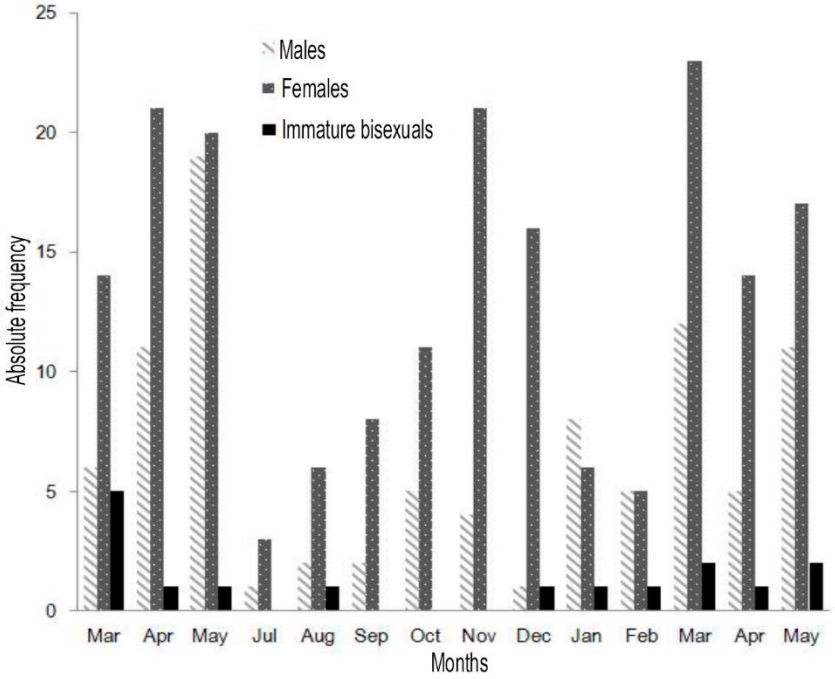

Figure 3. Absolute frequency of *M. rosacea* males (n = 93), females (n = 185), and immature bisexuals (n = 15) from March 2014 to May 2015.

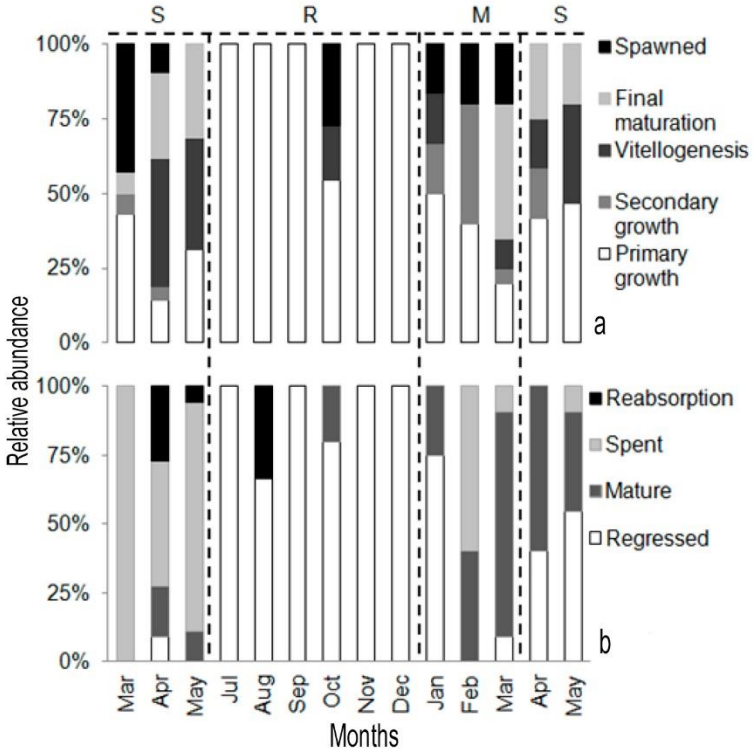

Figure 4. *M. rosacea* reproductive cycle. Relative abundance of the different stages in a) females and b) males during March 2014 to May 2015. S: spawn, R: rest stage, M: mature.

There was a negative correlation between GSI and temperature in males as well as females. This inverse relationship indicated that the GSI decreased at temperatures, whereas it increased at low temperatures. There was no correlation between HSI, CF, and temperature (Table 2).

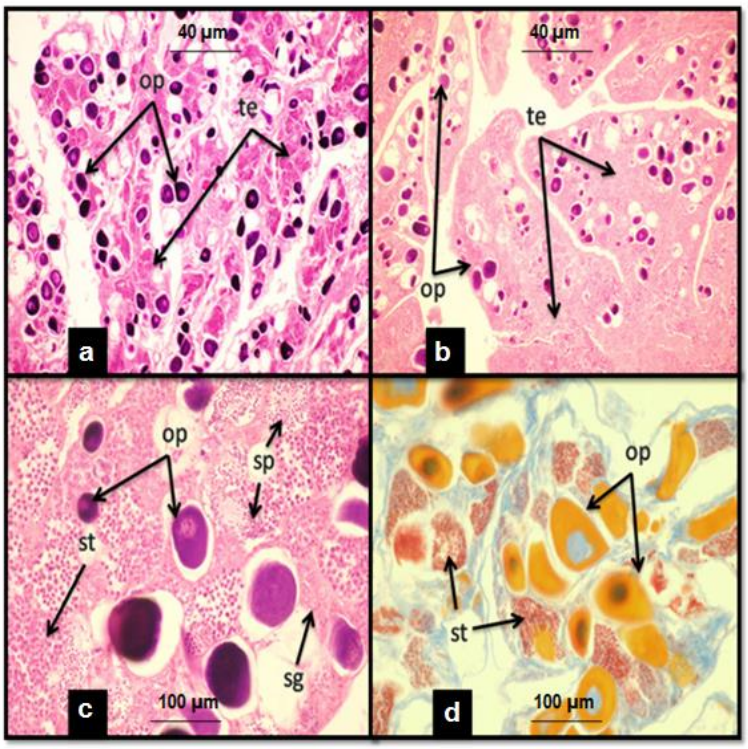

Figure 5. *M. rosacea* immature bisexuals: a-c) hematoxylin-eosin stain, and d) Mallory's Tricromic of stain. Op: primary oocyte, te: spermatic tissue, sp: spermatozoids, st: spermatocysts, sg: spermatogonia.

DISCUSSION

Leopard groupers *M. rosacea* reached a maximum size of 74 cm TL in Santa Rosalía (SR). The females were larger than the males, who did not surpass 68 cm TL. This size difference does not necessarily imply that this species presents sexual dimorphism, as has been proposed by Estrada-Godínez *et al.* (2011). These authors reported that males were larger than females (65 cm TL maximum size) in La Paz Bay (LPB; 400 km from our study site). However, Erisman *et al.* (2008) found that males and females achieved similar maximum sizes to the ones found in the present study in Bahía de Los Ángeles and Loreto; however, they did not report sexual dimorphism in *M. rosacea* based on size.

As reported previously in other studies (Kiewek-Martínez, 2004; Erisman *et al.*, 2008; Kiewek-Martínez *et al.*, 2010; Estrada-Godínez *et al.*, 2011), 15 individuals were cataloged as immature bisexual. It is presumed that immature bisexual individuals are females that develop spermatic tissue. However, as no previous maturity signal of either sex was observed, we consider that this is a gonochoric species that can present this immature bisexual stage. There is no evidence to date demonstrating the opposite, as has been reported for other serranid species classified as hermaphrodites (Erisman *et al.*, 2008).

Erisman *et al.* (2008) observed that immature bisexual individuals were smaller than mature males and females, and concluded that males derived from females. In the present study, according to histologic evidence, we found primary and secondary males, due to the presence of males measuring less than immature bisexual individuals without evidence of having gone through an immature bisexual stage.

The population sex ratio was 2:1 females to males, similar to what was found by Estrada-Godínez *et al.* (2011) for this same species in LPB. These authors reported that a sex ratio consisting of females being more abundant than males is commonly observed in congeners of this family. Differences in sex ratio also occurred in October (5.3 females: 1.0 males) and November (16.0:1.0 females to males).

According to McGovern *et al.* (1998), these variations occur in species forming reproductive aggregations, where males are captured at a higher rate than females, which would explain the decrease in males in the months following reproduction, as has been reported for *Mycteroperca microlepis*. These variations in sex ratios were also attributed to behavior; it was observed that in *M. microlepis* males separated

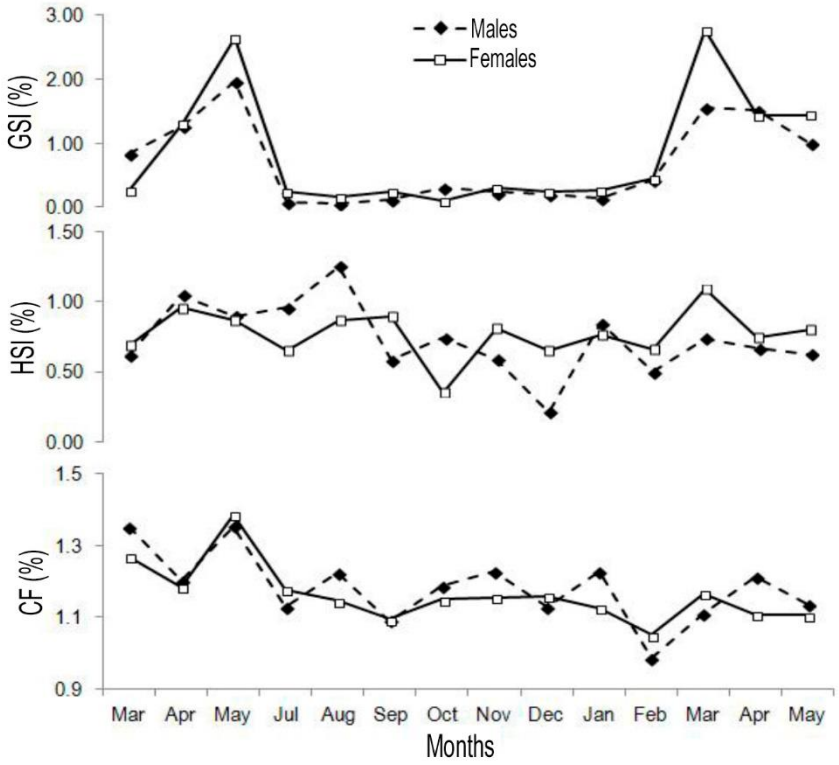

Figure 6. Monthly variation from March 2014 to May 2015. *M. rosacea* males and females. GSI: Gonadosomatic index, HSI: Hepatosomatic index, CF: Condition factor.

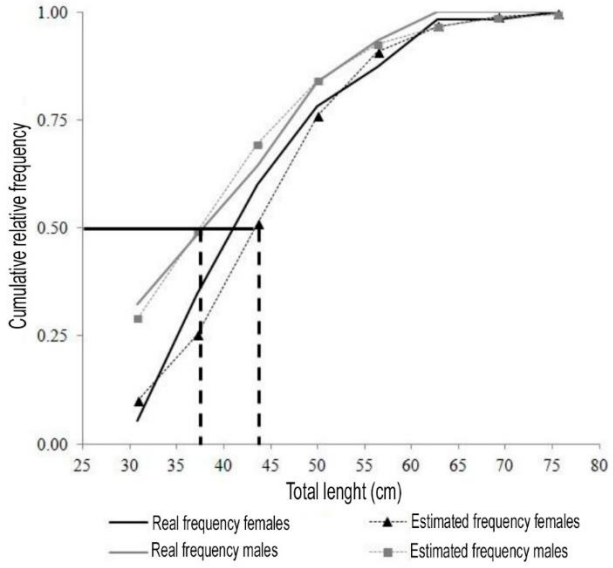

Figure 7. The logistic curve of cumulative relative frequency as a function of size class for females and males of *M. rosacea* (L_{50} females = 43.4 cm and L_{50} males = 37.4 cm).

from the females and became solitary after the reproductive event, migrating to deeper areas (50-90 m), while females moved to shallow areas (~30 m). This behavior could occur in *M. rosacea*, although it has not been reported yet. Given the behavioral and reproductive strategy of *M. rosacea*, it is probable that the sex ratio is skewed towards females due to fishing pressure or to sexual segregation, as has been reported for *M. microlepis*.

An essential part of reproduction is food availability, as individuals need to obtain the necessary energy for this event, and could be reflected by somatic indexes. The GSI attained its maximum values from March to May in 2014 and 2015, indicating the occurrence of the reproductive season. During this time the histologic cuts of the gonads showed the stages of highest gonadal development (vitellogenesis and final maturation), whereas the minimum values indicated the resting season (primary growth). Estrada-Godínez *et al*. (2011) also observed this pattern, with maximum index values coinciding with the most advanced gonad developmental stage, which indicates that the GSI is an ideal index to determine the time of sexual maturity.

The HSI recorded cyclic changes in liver weight due to lipid accumulation. The liver is also responsible for the production of vitellogenin, which facilitates the process of vitellogenesis in the oocytes and can be considered an organ of energy storage that can be used to measure the reserves available to the individual. This index should, therefore, have an opposite trend than the GSI (Saborido-Rey, 2008).

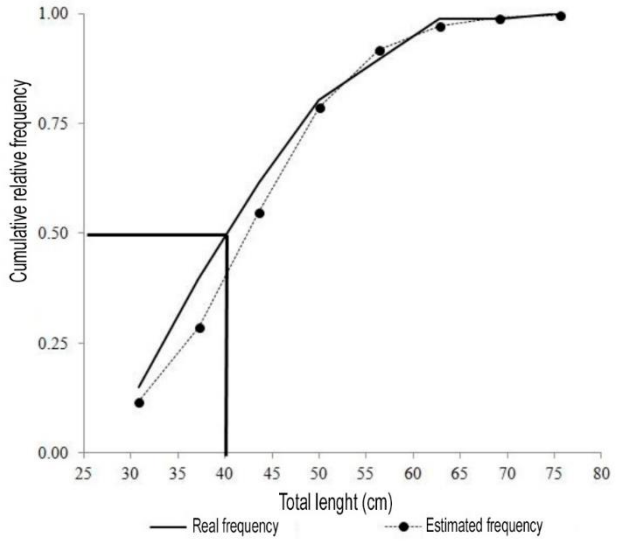

Figure 8. The logistic curve of cumulative relative frequency as a function of size class for *M. rosacea* (L_{50} = 40.8 cm).

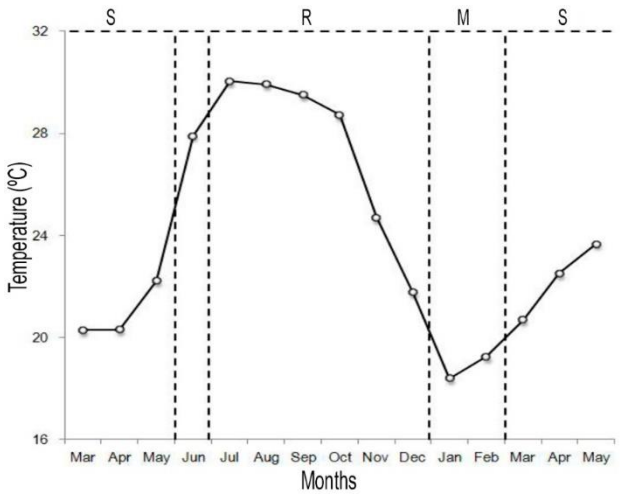

Figure 9. Superficial sea temperature variations from March 2014 to May 2015 related to the reproductive cycle of *M. rosacea*. S: spawn, R: resting, M: mature.

In this study no pattern was detected in the HSI that would indicate the reproductive season, and although Estrada-Godínez *et al*. (2011) observed higher values during the reproductive season, they attributed this to high protein concentration and accumulation of triglycerides and cholesterol, finding thus a positive correlation between GSI and HSI, which differs with what was found in the present study. According to energy transference, it should be expected that there would be a negative correlation between HSI and GSI. We did not find this correlation, and we assume that *M. rosacea* is not a species for which energy accumulation plays a vital role in the reproductive process as has been detected in other teleost fish (Saborido-Rey, 2008).

The CF reflects the energy available to the organism, and similarly to the HSI, it was not correlated to the GSI, as was reported by Estrada-Godínez *et al*. (2011), who did not observe variations in this index during the year. It seems that contrary to other species that fast before reproduction (Saborido-Rey, 2008), *M. rosacea* feeds year-round regardless of the maturity stage in which it is found, as was also postulated by Estrada-Godínez *et al*. (2011).

As confirmed by the study by Pérez-Rojo (2016), it was determined that *M. rosacea* is an active predator with broad trophic plasticity in the SR area. Despite being considered an ichthyophagous predator with a marked preference for sardines (*Sardinops caeruleus*) during the cold season, it can alternate its diet to consume mainly euphausiids (*Euphasia distinguenda*) during the warm season. These two prey species are abundant during the cold and warm season, respectively. It was confirmed that *M. rosacea* does not require storage of large energy reserves; this was validated by the HSI and CF values, which did not present notable variations over an annual cycle.

The energy transference required for the reproductive event depends on external factors that indicate when the most favorable conditions are occurring. Temperature played an essential role in gametogenesis, as spawning in SR occurred at between 20 and 23ºC, similar to what was reported by Estrada-Godínez *et al*. (2011) for the same species in LPB (21-25ºC).

Kiewek-Martínez *et al*. (2010) and Estrada-Godínez *et al*. (2011) stated that temperatures above 25ºC were linked with the end of spawning in leopard grouper, and with the highest percentage of individuals in atresia. There are no data for June, and it is presumed that the highest number of atresias would occur in this month because the temperature in June 2014 was 27.9ºC; therefore, this month is when the post-spawn stage could occur. It was observed that *M. rosacea* differs from other species for which the beginning of gametogenesis and spawning occur at the highest temperatures (Kiewek-Martínez, 2004).

Accordingly, with the reproductive cycle established for *M. rosacea* in the area of SR and contrary to what was found by Estrada-Godínez *et al*. (2011) for BLP, there were temporal differences in the cycle stages. The resting stage for individuals in SR started in July, whereas it started one month later in BLP, but it ended in December for the two populations. Gonadic maturation occurred in January and February, although in BLP it occurred two months later (March to April), in a subsequent year it occurred in January and February, as was reported in the present study.

Table 2. Spearman correlation between somatic indexes and average temperature. GSI: gonadosomatic index, HSI: hepatosomatic index, CF: condition factor.

		Temperature	Male	Female
GSI	Temperature		-0.454945	-0.415385
	Male	-0.454945		0.846154
	Female	-0.415385	0.846154	
HSI	Temperature		0.226374	-0.015385
	Male	0.226374		0.305495
	Female	-0.015385	0.305495	
CF	Temperature		-0.160440	-0.085714
	Male	-0.160440		0.476923
	Female	-0.085714	0.476923	

Spawning lasted two months (May-June) in BLP, whereas it lasted three months (March-May) in SR, with the highest percentage of spawning females in 2015. These differences could be due to the dynamics of the GC compared with BLP, as BLP has particular hydrographic characteristics (Obeso-Nieblas *et al.*, 2014), due to the intrusion of oligotrophic waters in summer characterized by high temperatures and low nutrients, which cause a delay in BLP compared with the ocean dynamics within the GC (Obeso-Nieblas *et al.*, 2014).

There was a low percentage of individuals presenting hydrated oocytes or with post-ovulatory follicles in the reproductive season, which suggests that the sampling site is not an important reproductive area and that *M. rosacea* could be migrating towards the southern end of the sampling area. Erisman *et al.* (2007) and Aburto-Oropeza *et al.* (2008) suggest that this species could move to Bahía de Loreto due to the vast number of individuals observed at that location. Despite Bahía de Loreto being 175 km south of SR, reproductive migrations of considerable distance have been reported for *M. microlepis* (McGovern *et al.*, 1998), which could also be occurring with *M. rosacea*.

Males at the spermiated stage were observed from March to May 2014 and from January 2015, which indicated that the beginning of male spermatic cell maturation occurs a few months before female maturation, similar to what was observed by Estrada-Godínez *et al.* (2011).

We were able to determine the size at first maturity using the gonadal maturation degree. According to Aburto-Oropeza *et al.* (2008), the leopard grouper needs 14 years to duplicate its population size, and the size of first catch occurs at approximately 2.5 years. In the present study, we obtained a size at first maturity of 40.8 cm TL in the coastal area of SR, which differs from the size reported by Aburto-Oropeza *et al.* (2008) of 42 cm for the GC. It should be pointed out that these authors did not specify the method used to determine size at first maturity so that the estimation in our study is the first in which this determination was carried out systematically with an adequate number of individuals. There was no marked difference between the two estimates despite there being methodological uncertainties in the calculation made by Aburto-Oropeza *et al.* (2008), and despite the difference in the years between that study and ours, so that we can infer that the population is still healthy with regards to fisheries.

Female size at first maturity was 42.4 cm, whereas male size at first maturity was 37.3 cm, denoting possible higher fishing pressure on males of this species. *M. rosacea* reproductive aggregations are formed with one female surrounded by approximately 40 males (Aburto-Oropeza *et al.* 2008). The probability of males making up the largest share of the catch can lead to higher fishing pressure and to a need for males to mature at smaller sizes. However, some populations are characterized by females having a larger size at first maturity than males (Saborido-Rey, 2008).

According to what was observed during this study, the reproductive event is influenced by food availability as well as by sea surface temperature, which indicates that the environmental characteristics could modify the timing or intensity of reproduction. Temperature allows the synchronization of optimum environmental conditions and induces the final maturation and spawning of gametes (Estrada-Godínez *et al.*, 2014).

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