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Reproductive biology of the Crimson jobfish *Pristipomoides filamentosus* (Valenciennes, 1830) landed along the southern coast of Kerala, India

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Abstract

The crimson jobfish *Pristipomoides filamentosus* is an important food fish belonging to the family Lutjanidae, distributed widely throughout the Indo-Pacific region, with high consumer demand and potential as a candidate species for farming. Reproductive biology of *P. filamentosus* was studied from 594 specimens (317 females and 277 males) collected from Vizhinjam fish landing centre on the south-west coast of India during October 2022-March 2023. The fork length (FL) of the fish ranged from 19.1 to 75.1 cm in females and 19.7 to 76.5 cm in males and the body weight (BW) ranged from 103.5 to 4276.5 g in females and 130.1 to 4346.1 g in males. The sex ratio (male: female) was 1:1.1, reflecting a marginal dominance of females in most of the months and size ranges. For females, the mean length at first maturity (Lm_{50}) was estimated at 36.6 cm FL and for males, 35.5 cm FL. GSI showed maximum values for both females (1.73) and males (2.52) in January and February, respectively. Spawning-capable females and males were dominant during February (62.2%) and January (59.4%). Asynchronous ovarian development demonstrated multiple spawning with two distinct modes (375-404 m and 465-494 m) in the oocyte diameter distribution. Fecundity increased with fork length, body weight and ovary weight, which ranged from 151,573 to 724,856 eggs, with an average of 432,983 eggs. Based on the analyses of maturity phases and histological studies, immature, developing, spawning-capable, regressing, and regenerating maturity phases for both sexes are described. Globally, information on the reproductive biology of *P. filamentosus* is meagre. Results of the present study could contribute to broodstock development and effective management of its fishery in the region.

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Introduction

Snappers are generally inhabitants of steep coral and rocky reefs (Parrish, 1987) and are distributed throughout the tropical, subtropical seas of the Indo-Pacific, Hawaiian Archipelago, Central Western Pacific Ocean, south along the North-western and North-eastern Australia (Allen, 1985; Kramer *et al.,* 1994; Hardman-Mountford *et al.,* 1997). Around 49 species of snappers have been reported from Indian waters (Nair *et al.,* 2014). Only fifteen species commonly land along the coast of Kerala and the crimson jobfish *Pristipomoides filamentosus* (Valenciennes, 1830) is the dominant one among these (Wilson *et al.,* 2019). The genus *Pristipomoides* consists of 11 species (*P. aquilonaris*, *P. argyrogrammicus*, *P. auricilla*, *P. filamentosus*, *P. flavipinnis*, *P. freemani*, *P. macrophthalmus*, *P. multidens*, *P. sieboldii*, *P. typus* and *P. zonatus* (Anderson, 1986). Most species in this group are commercially important, and many have either mariculture or ornamental potential (James *et al.,* 1996). *P. filamentosus* is highly preferred for its high-quality white meat (Roul and Pradhan, 2019). *P. filamentosus* lives up to 44 years (Andrews *et al.,* 2012), reaching a maximum length of 100 cm (Anderson, 1986). It has been reported from deep waters up to 360 m (McAllister *et al.,* 1992), weighing up to 9 kg (Manooch, 1987; Randall *et al.,* 1998). It is a

gonochoristic, multiple batch spawner with pelagic eggs (Grimes, 1987). The estimated landing of snappers in 2022 was 11,512 t along the Indian coast (CMFRI, 2023).

Understanding the reproductive biology of a species is crucial for providing scientific advisories for fisheries management, assessing stock status and implementing effective fishery management practices. This knowledge, as emphasised by various studies (Everhart *et al.,* 1975; Unver and Saraydin, 2004; Al-Marzouqi *et al.,* 2011), is essential for decision-making, ensuring sustainable exploitation, and preserving the long-term health of fish populations. In Hawaii, *P. filamentosus* is a part of Hawaii 'Deep-7' bottom fish species complex in marine protected areas and is managed by total allowable catch regulations (Friedlander *et al.,* 2014; Sackett *et al.,* 2014; Luers *et al.,* 2017). Regarding the conservation status, *P. filamentosus* is placed on the IUCN Red List in the "Least Concern" (LC) category (Acero, 2010; Russell *et al.,* 2016 a, b, c).

The only report available on the reproductive biology of *P. filamentosus* from the Asian region is by Uehara *et al*. (2018) Japan. Hence, a detailed study on the reproductive biology of *P. filamentosus* from the south-west Indian coast is attempted here.

Materials and methods

A total of 594 specimens were collected from the landings of outboard non-mechanised boats operating from the Vizhinjam fishing harbour (8°22'42.3"N and 76°59'27.9"E) on the southern coast of Kerala, India (Fig. 1). Landings of *P. filamentosus* are seasonal, from October to March every year at Vizhinjam and there is no targeted fishery for this species for the rest of the months. The samples were collected from October 2022 to March 2023. The specimens (Fig. 2) were placed in insulated ice boxes and transported to the laboratory of the Vizhinjam Regional Centre of ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Vizhinjam, India.

The samples were thoroughly washed before recording the body colouration in fresh condition and photographed with a digital camera (Canon Ixus 185, Japan). Fork Length (FL) was measured to the nearest 0.1 cm and body weight (BW) was measured to the nearest 0.1 g. After dissecting the fish sample, the sexes were determined from visual observation of the gonads. The gonads were weighed using an electronic weighing balance to the nearest 0.01 g. Length-wise and month-wise ratios between males and females were determined and the significance was evaluated using the Chi-square test (Zar, 1984). Length at first maturity, Lm_{50}, defined as the fork length at which 50% of the individuals attain sexual maturity, was estimated by logistically fitting the proportion of matured fish in different size classes as described by King (1995) following the formula: P=1/1+exp (a + bFL), where 'P' is the predicted mature proportion, 'a' (intercept) and 'b' (slope) are coefficients of the logistic equation and 'FL' is the fork length (cm).

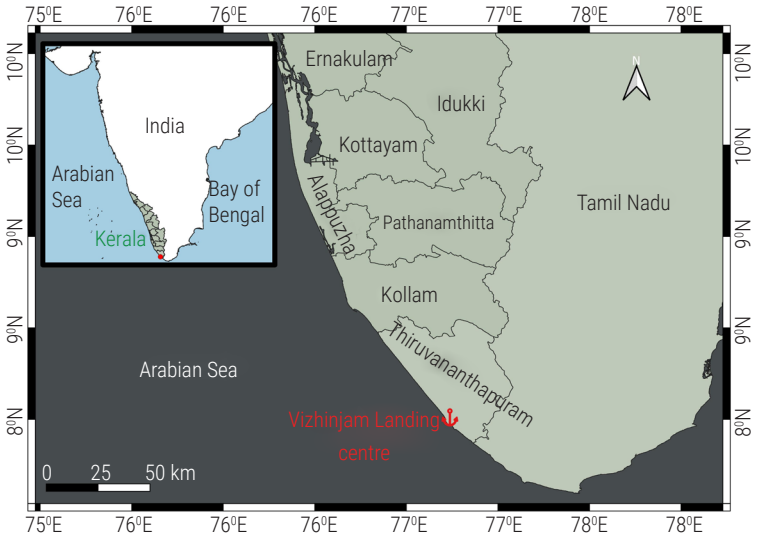


Fig. 1. Sampling location of *P. filamentosus*

Fig. 2. *P. filamentosus* (FL - 37.5 cm)

The gonadosomatic index (GSI) was determined from the gonad weight (GW) and body weight (BW) of the fish using the equation GSI = (GW/BW)/100. Gonadal maturity phases were determined using the standards defined by Brown-Peterson *et al*. (2011). Ovaries and testes were classified into five stages: immature, developing, spawning capable, regressing and regenerating. Fecundity (F) was determined for 30 spawning capable females, in the range of 29.5 to 43.5 cm FL by gravimetric method described following Hunter and Macewicz (1985) and Murua *et al*. (2003). The formula used to estimate the number of oocytes was: BF = {[∑i (Oi/Wi)]/n}*Wo, where 'Oi' is the sub-sample count, 'Wi' is the sub-sample gonad weight, 'n' is the number of sub-samples, and 'Wo' is the weight of the ovary.

The relationship between fecundity and other variables like FL and BW was calculated by log transformation of the empirical formula $F = aX^b$, where 'F' is the fecundity, 'a' is a constant, 'X' is the variable (FL and BW) and 'b' is the correlation coefficient (Zupa *et al*., 2013).

For histological analyses, subsamples of gonads (3-5 mm thickness) from the anterior, middle and posterior portions were fixed in 10% neutral buffered formalin (NBF) for a day. These were then dehydrated in ascending series of alcohol, cleared in xylene, embedded in paraffin wax and sectioned transversely into 5 µm thickness following standard procedures (Gray, 1964). The sections were mounted on glass slides and stained with haematoxylin and eosin (Mokhtar, 2018). Ova diameter was measured using the procedures outlined by Clark (1934) and Prabhu (1956). A total of 50 ovaries at different maturity phases were analysed, and from each ovary, the diameters of 100 ova were measured. Oocyte development within the ovary was studied and frequency polygons of oocyte diameter in different phases of maturity were drawn.

Results and discussion

Sex ratio

A total of 594 fish were analysed, including 317 (53.37%) females and 277 (46.63%) males. In females, the range of FL was from 19.1 to 75.1 cm and the range of BW was from 103.5 to 4276.0 g. In males, FL ranged from 19.7 to 76.5 cm and the BW ranged from 130.1 to 4346.1 g. The overall male: female sex ratio was 1:1.1, with significant differences (χ^2=2.693; p>0.05) indicating the dominance of females in the landings. Large deviations in the sex ratio of *P. filamentosus* up to 1:3.5 were observed within specific length classes (19.5-24.4 cm FL) and months (March) up to 1:3.3 (Table 1). Similar instances have been reported in *P. auricilla* and *P. flavipinnis* (Kami, 1973). An increased percentage of females in the sex ratio has also been reported for *P. filamentosus* from the Main Hawaiian Islands (Leurs *et al.,* 2017) and for four species of snappers (*P. filamentosus*, *P. sieboldii*, *Etelis coruscans* and *Paracaesio caerulea*) in the Okinawian waters, Japan (Uehara *et al.,* 2018). The sex ratio can be related to the status of the fish population and sustainability (Fernandes *et al.,* 2022).

Length at first maturity (Lm_{50})

The minimum length at maturity of *P. filamentosus* female and male fish were recorded at FL 23.4 cm and 21.5 cm, respectively. The estimated Lm_{50} was 36.6 cm FL for females and 35.5 cm FL for males (Fig. 3). The Lm_{50} reported for female *P. filamentosus* was 40 cm in FL in Hawaii (Ralston and Miyamoto, 1983), 42.5 cm FL in the North-western Hawaiian Islands (Kikkawa, 1984) and 38.8 cm FL in Tongen Seamounts (Mees and Rossouw, 1997). The Lm_{50} of *P. filamentosus* was reported as FL 40.7 cm for females and

Table 1. Length-wise and month-wise sex ratio of *P. filamentosus*

FL Class (cm)	Number of Males (M)	% (M)	Number of Females (F)	% (F)	M/F	Chi-square (χ^2) value	Probability (p value)
19.5 ⊦	09	21.95	32	78.05	1:3.5	12.902	0.003*
24.5 ⊦	26	37.14	44	62.86	1:1.7	4.628	0.031*
29.5 ⊦	12	30.00	28	70.00	1:2.3	6.400	0.011*
34.5 ⊦	95	60.13	63	39.87	1:0.7	6.481	0.010*
39.5 ⊦	66	44.59	82	55.41	1:1.2	1.729	0.188
44.5 ⊦	42	48.28	45	51.72	1:1.1	0.103	0.747
49.5 ⊦	06	75.00	02	25.00	1:0.3	2.000	0.157
54.5 ⊦	09	75.00	03	25.00	1:0.3	3.000	0.083
59.5 ⊦	03	33.33	06	66.67	1:2.0	1.000	0.317
64.5 ⊦	02	40.00	03	60.00	1:1.5	0.200	0.654
69.5 ⊦	02	40.00	03	60.00	1:1.5	0.200	0.654
74.5 ⊦	05	45.45	06	54.55	1:1.2	0.090	0.763
Total	277	46.63	317	53.37	1:1.1	2.693	0.100
Month							
October	13	32.50	27	67.50	1:2.1	4.900	0.026*
November	34	61.82	21	38.18	1:0.6	3.072	0.079
December	99	55.93	78	44.07	1:0.8	2.491	0.114
January	56	69.14	25	30.86	1:0.5	11.864	<0.001*
February	66	32.67	136	67.33	1:2.1	24.257	<0.001*
March	09	23.08	30	76.92	1:3.3	11.307	<0.001*
Total	277	46.63	317	53.37	1:1.1	2.693	0.100

FL 34.3 cm for males in the Main Hawaiian Islands (Luers *et al.,* 2017) and FL 35.7 cm for females and FL ≥ 20 cm for males in the Okinawan waters, Japan (Uehara *et al.,* 2018). Since *P. filamentosus* is a species with a longer lifespan and relatively bigger size at initial maturity, it may be more vulnerable to overfishing than species with smaller bodies, early maturation, and shorter lifespans, as explained by Wiedmann *et al.* (2014). The majority of tropical marine fishes mature and spawn at an early age, usually during the first or second year of their life (Qasim, 1973). According to Uehara *et al*. (2018), *P. filamentosus* attains sexual maturity within the first two years, despite its long life span. Understanding the length at first maturity is important for fishery management since it provides information on the status of spawning stock biomass (Rajesh *et al.,* 2020).

Gonadosomatic index (GSI) and maturity phases

The mean GSI values for females varied across months, ranging from 0.35 to 1.73. The peak values were observed in January (1.73±0.26), February (1.31±0.14) and March (1.05±0.13), coinciding with the frequency of spawning-capable females (Fig. 4). Significantly higher mean GSI values were also observed in these months. The occurrence of specimens with immature ovaries (Phase I) was high in the month of February (46.1%), followed by October (44.4%). Developing ovaries (Phase II) were more prominent in March (45.0%), while the highest percentage of specimens with spawning-capable ovaries (Phase III) occurred in January (62.2%), followed by December (30.7%) and March (30.0%). Regressing ovaries (Phase IV) were more common in November (35.7%) and December (28.8%), while there were more regenerating ovaries (Phase V) in November (21.4%) and December (21.1%).

Mean GSI values for males also varied across months, ranging from 0.36 to 2.52. The peak values were observed in February (2.52±0.28), January (2.36±0.20) and March (2.26±0.37), coinciding with the frequency of spawning-capable males. (Fig. 5). As in the case of females, significantly higher mean GSI values were also observed in these months. Occurrence of specimens with immature testes (Phase I) was high in October (66.6%). Developing testes (Phase II) were more prominent in March (50.0%), while the highest percentage of specimens with spawning-capable testes (Phase III) occurred in January (59.4%), followed by February (56.4%). Regressing testes (Phase IV) were common in January (24.3 %) and

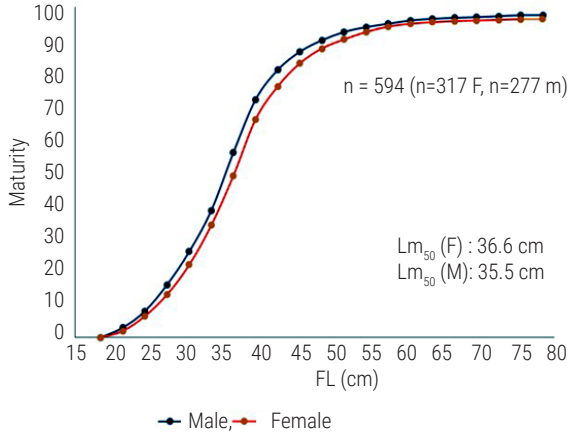


Fig. 3. Length at first maturity of females (F) and males (M) of *P. filamentosus*

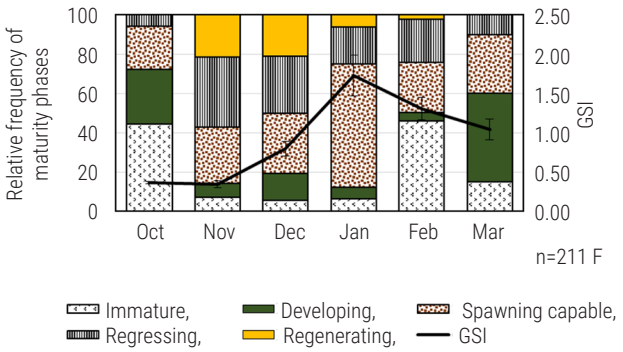


Fig. 4. Month-wise GSI and relative frequency of maturity phases in female *P. filamentosus*

December (19.7%). There were more regenerating testes (Phase V) in December (24.2%) and November (21.7%).

The present study validated the reproductive cycle of *P. filamentosus* caught from the southern coast of Kerala, indicating a peak spawning period for *P. filamentosus* during January-March.

The mean monthly GSI values were above 1.0 except for the spawning period, with a peak recorded in January for females and February for males. The mean length-wise GSI value observed was above 1.5 in both sexes at 64.5-84.5 cm FL. Kikkawa (1984) studied the relationship between GSI and maturity phases of *P. filamentosus* from the North-western Hawaiian Islands and studies of Uehara *et al.* (2018) from Okinawan waters suggested that the species has a protracted spawning season.

Fecundity

The fecundity determined for spawning-capable females was between 151,573 and 724,856. The relative fecundity ranged from 265 to 943 ova g^{-1} with an average of 682 ova g^{-1}. In the analysis of the relationship between fecundity (F) with fork length (FL) (cm) and body weight (BW) (g), a moderate positive correlation was observed (R^2 = 0.35 0.49, respectively) for all. The relationship between FL and BW was exponential (Fig. 6a, b). The fecundity estimates of *P. filamentosus* from North-western Hawaiian Islands ranged from 478,000 to 1,462,000 in fish of FL 48.7 - 76.3 cm (Kikkawa, 1984). Species with larger sizes (35.6-55.5 cm in standard length), such as *P. multidens*, *P. typus* and *P. filamentosus*

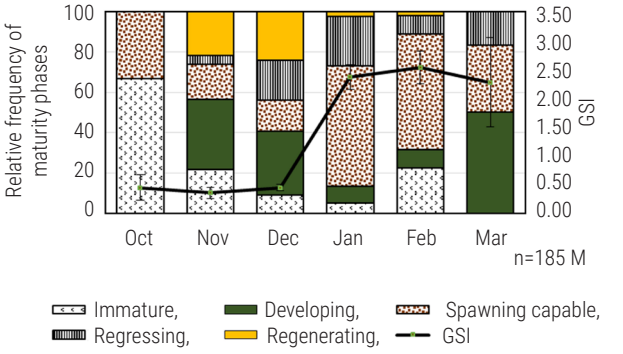


Fig. 5. Month-wise GSI and relative frequency of maturity phases in male *P. filamentosus*

had fecundity ranging between 490,000 and 2,770,000 (Grimes, 1987). In the present study, the smallest fish measuring 29.5 cm FL had a fecundity of 246,280 eggs. However, the lowest number (151,573) of eggs was recorded from a fish of FL 35.5 cm and the highest number (724,856) of eggs from a fish of FL 41.0 cm. Lutjanids, especially the deep-sea snappers in island habitats are considered to have a high fecundity rate (Grimes, 1987). According to Barbieri and Lowerre-Barbieri (2011), the reproductive success of a fish population mainly depends on the fecundity of the females and the survival rate of their offspring. Kikkawa (1984) estimated a positive relationship between fecundity, fork length (R^2 = 0.9) and body weight (R^2 = 0.65) in *P. filamentosus*. Nanami (2011) reported a positive relationship between fecundity and FL in 16 females with mature ova for oblique-banded snapper (*P. argyrogrammicus*). In the current study, a moderately positive linear relationship was observed between fecundity and fork length, as well as fecundity and body weight as assessed from 30 females of *P. filamentosus*.

Ovarian development in *P. filamentosus*

Immature ovary

Fishes (n=87) with immature ovaries ranged from 19.1 to 32.5 cm in FL and 103.5 to 602.4 g in BW and the GW ranged from 0.1 to 2.9 with a mean GSI value of 0.26 (0.15). Morphologically, the immature ovaries were pinkish, elongated and translucent (Fig. 7a, b). Blood vessels were visible only through a microscope. Ovarian biopsy revealed honeycomb-like transparent primary oocytes (Fig. 7c). In histological observation, oogonia were small and clearly stained with haematoxylin and the maximum size observed was 100 µm (Fig. 7d). Oogonia (OG) were honeycomb-like and primary growth (PG) ova had large nuclei. The ovary wall (OW) was very thin, with little space between the ova and moderately developed lamellae with clear interlamellar space (ILS).

Developing ovary

Fish (n=41) with developing ovaries were mainly within the size range of 21.0 to 37.4 cm in FL, 192.3 to 781.0 g in BW and the GW ranged between 0.7 g and 3.7 g with a mean GSI value of 0.59 (0.40). The ovaries were yellowish in colour, slightly enlarged, highly vascularised and occupied 1/3 to 2/3 of the body cavity (Fig. 8a, b). In biopsy, ova were mainly in the secondary vitellogenic (Vtg 2) stage and the diameter ranged between 105 and 309 µm (Fig. 8c). Histology revealed three types of ova in this stage: Primary growth oocytes (PG), Primary vitellogenic (Vtg 1) and Secondary vitellogenic (Vtg 2). The presence of cortical alveoli (CA) indicated the beginning of maturation (Fig. 8d).

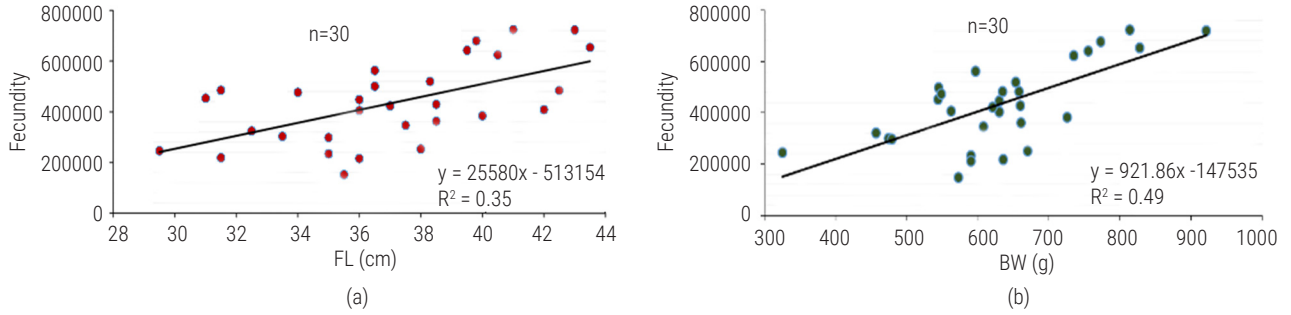


Fig. 6. Relationships between fecundity to (a) FL and (b) BW of *P. filamentosus*

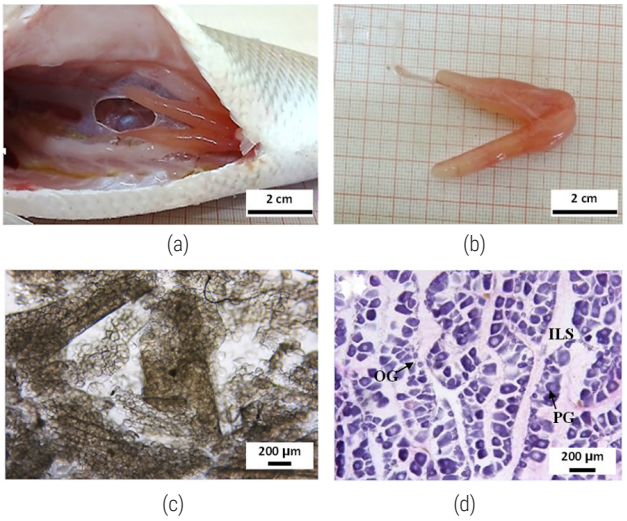


Fig. 7. Immature ovary of *P. filamentosus*; (a) *In situ* observation; (b) *Ex-situ* observation; (c) Ovarian biopsy; (d) Histological observation showing the presence of oogonia (OG) and primary growth (PG) oocytes

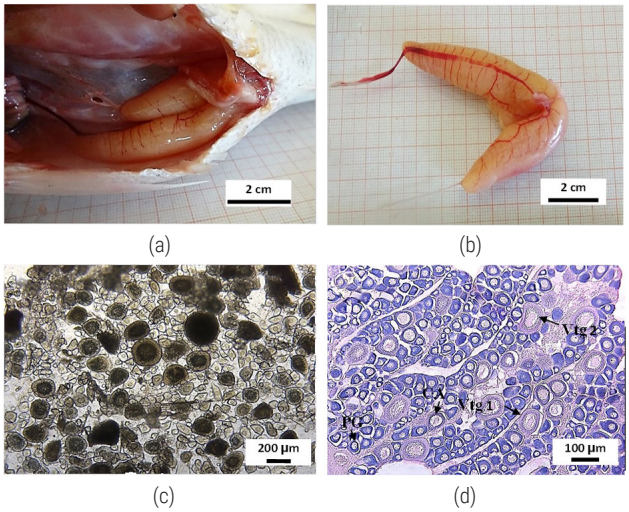


Fig. 8. Developing ovary of *P. filamentosus*; (a) *In situ* observation; (b) *Ex situ* observation; (c) Ovarian biopsy; (d) Histological observation with the presence of primary growth oocytes (PG), cortical alveoli (CA), primary vitellogenic (Vtg 1) and secondary vitellogenic (Vtg 2) oocytes

Spawning-capable ovary

Fish (n=95) with spawning-capable ovaries ranged from 29.5 to 75.1 cm in FL, 325.3 to 4276.0 g in BW and GW ranged from 5.9 to 104.0 g with a mean GSI value of 2.19 (1.45). The ovaries were large and yellowish-orange in colour with conspicuous blood vessels. More than 2/3 of the body cavity was occupied by the ovary (Fig. 9a, b). In biopsy samples, most of the ova were in the size range of 375 to 494 µm in diameter and were visible without a microscope (Fig. 9c). Histological examination showed the presence of both CA and PG. Most of the gonads were at the tertiary vitellogenic (Vtg 3) stage with germinal vesicle migration and post-ovulatory follicles (POFs) were also observed (Fig. 9d).

Regressing ovary

Fish (n=68) with regressing ovaries had a size range from 27.5 to 55.1 cm in FL and 328.3 to 2445.5 g in BW and the GW ranged from 4.5 to 18.5 g with a mean GSI value of 1.60 (0.76). The ovaries were transparent and reddish yellow in colour with barely visible blood vessels (Fig. 10a, b). Tertiary vitellogenic (Vtg 3) ova and enlarged post-ovulatory follicles (POFs) were observed. The ova size ranged between 255 and 645 µm (Fig. 10c) and hydrated (H) and atresia (A) stages were also present. Histological sections (Fig. 10d) also showed some vitellogenic ova (Vtg 1, Vtg 2) and cortical alveolar ova (CA).

Regenerating ovary

Fish (n=26) with regenerating ovaries with FL ranged from 37.0 to 42.5 mm, BW ranged from 667.7 to 1031.8 g and GW ranged from 0.8 g to 3.8 g with a mean GSI value of 0.27 (0.13). Ovarian lobes were medium-sized and dark red in colour. The ovaries were smaller (Fig. 11a, b) oogonia (OG), totally hydrated eggs (H), and primary growth ova (PG) could be seen in the biopsy. The egg size varied from 45 to 584 µm (Fig. 11c). Interlamellar space (ILS), thick

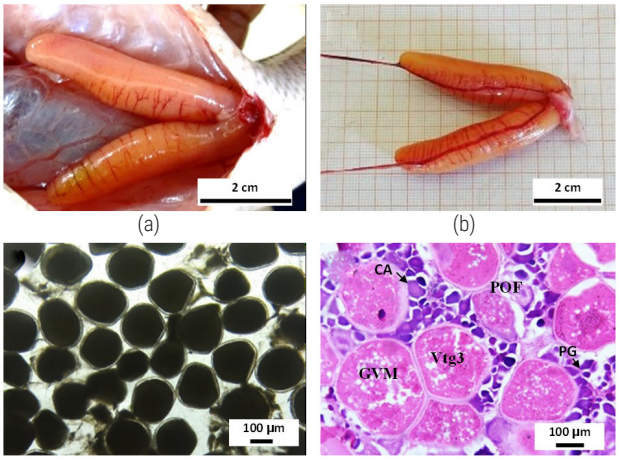


Fig. 9. Spawning capable ovary of *P. filamentosus*; (a) *In situ* observation; (b) *Ex situ* observation; (c) Ovarian biopsy; (d) Histological observation with the presence of primary growth oocytes (PG), Cortical alveoli (CA), Tertiary vitellogenic (Vtg 3), Germinal vesicle migration (GVM) and Post-ovulatory follicle (POF)

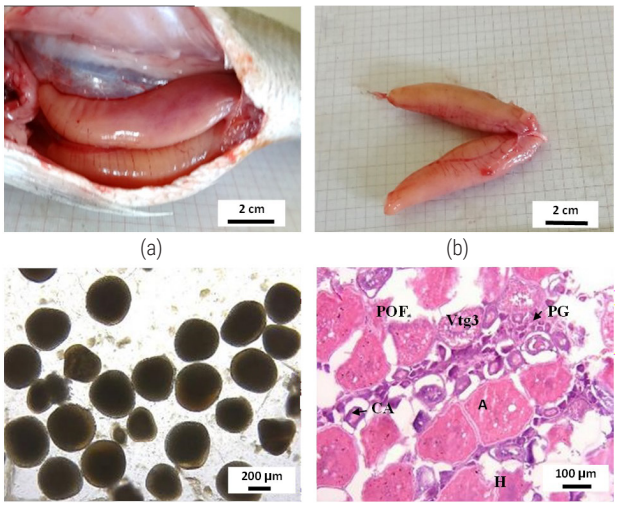


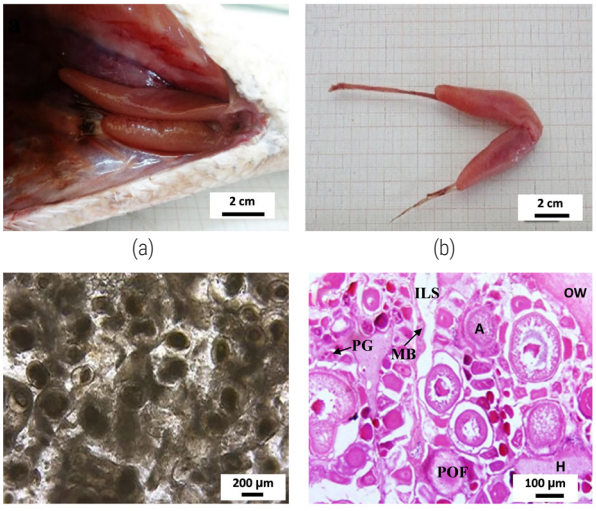
Fig. 10. Regressing ovary of *P. filamentosus*; (a) *In situ* observation; (b) *Ex situ* observation; (c) Ovarian biopsy; (d) Histological observation showing the presence of Primary growth oocytes (PG), Cortical alveoli (CA), Tertiary vitellogenic (Vtg 3), Post-ovulatory follicle (POF), Hydrated (H) and Atresia (A) stages

ovarian wall (OW), primary growth oocytes (PG), cortical alveoli (CA), atresia (A), hydrated (H) and degenerating post-ovulatory follicles (POFs) were present in histological observations (Fig. 11d).

Histological study of *P. filamentosus* gonads showed that they can spawn more than once, which is in line with what Luers *et al*. (2017) and Uehara *et al*. (2018) found. Studies on the development of the ovaries in related species, like *P. argyrogrammicus* off the coast of Ishigaki Island, Okinawa (Nanami, 2011) and *P. zonatus* from Guam (Schemmel *et al*., 2022), have also shown that these fish spawn more than once a year. Multiple oocyte maturation stages and post-ovulatory follicles (POFs) were found in this study during the spawning season, which is similar to what other studies have found.

Ova diameter

The diameter of the ova from representative samples of all stages of ovaries of *P. filamentosus* were measured (Fig. 12). In the immature ovary, most of the ova were within the range of 75-104 µm. Very few ova measuring 45-74 and 105-134 µm were also present. In the developing ovary, along with the ova of 75-104 µm, a batch of ova within the size range of 195-224 µm was observed as separate from the general batch of ova. A few large ova measuring 285-314 µm were also seen. Several groups of developing ova with diameters ranging from 45 to 554 µm were observed in the ripe ovaries of spawning-capable females, indicating an extended spawning period of *P. filamentosus*. The frequency polygon of the spawning-capable group indicated multiple spawning with two distinct modes (375-404 and 465- 494 µm) in the ova diameter. In regressing ovary, the mature ova measured 495-524 µm, forming a prominent mode and the larger ova measured 645-674 µm. In the regenerating ovary, several post-ovulatory follicles measuring 465-584 µm were observed while primary oocytes formed a mode at 75-104 µm. In the oocyte diameter frequency polygon, spawning-capable ovaries



(a) (b) (c) (d)

Fig. 11. Regenerating ovary of *P. filamentosus*. (a) *In situ* observation; (b) *Ex situ* observation; (c) Ovarian biopsy; (d) Histological observation showing the presence of primary growth oocytes (PG), Cortical alveoli (CA), Post-ovulatory follicle (POF), Hydrated (H) and Atresia (A) stages, Interlamellar spaces (ILS) and Muscle bundles (MB)

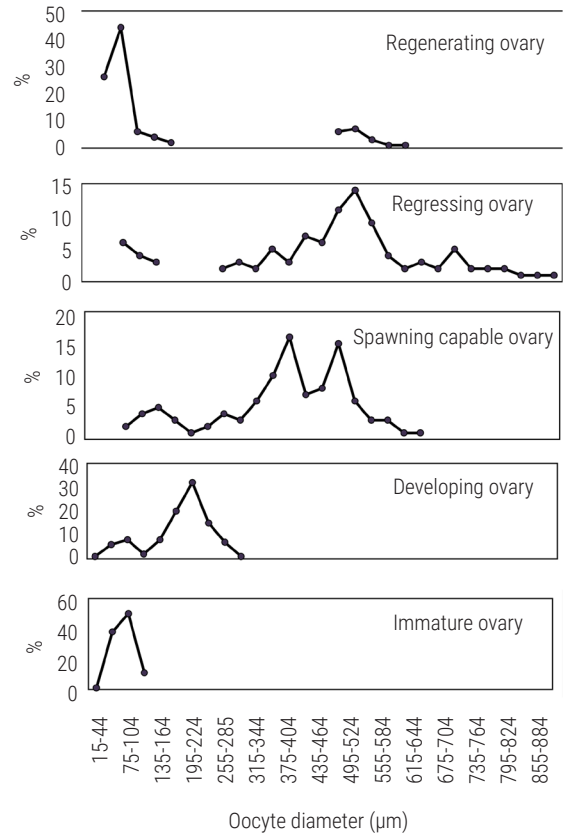


Fig. 12. Oocyte diameter (µm) frequency polygon of *P. filamentosus*

showing oocyte diameter progression with two distinct modes (375-404 µm and 465-494 µm) indicated *P. filamentosus* to be a multiple spawner. Multiple spawning is indicated by the presence of yolked eggs of varying sizes within a single mature ovary (Clark, 1934; De Silva, 1973; Brule *et al.,* 2004). In *P. filamentosus*, there were multiple modes indicating asynchronous ovarian development as reported in the case of *P. zonataus*, by Schemmel *et al*. (2022). In snappers, more than one spawning was reported in a single year (Grimes, 1987; Mees, 1993; Nanami, 2011; Luers *et al.,* 2017; Uehara *et al.,* 2018). Kikkawa (1984) reported an ova size range of 390-600 µm and 470-580 µm in the advanced developing stage and early ripe ovary respectively for *P. filamentosus*. Ramachandran *et al*. (2013) reported different stages of ova during the spawning season with diameters ranging from 110-810 µm in brown stripe snapper (*Lutjanus vitta*) from the south-west coast of India. Multiple spawning events were also reported in *P. multidens* (Min *et al.*, 1977).

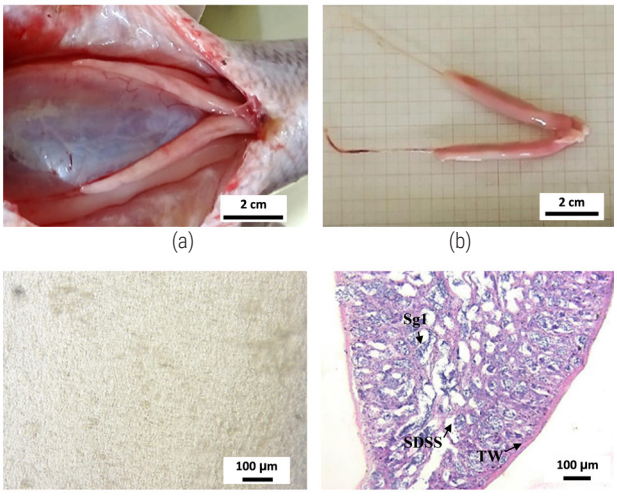
Testicular development in *P. filamentosus*

Immature testes

Fishes (n=44) with immature testes ranged from 19.7 to 34.0 cm in FL and 130.1 to 727.1 g in BW and the GW ranged from 0.4 to 1.1 g with a mean GSI value of 0.24 (0.16). The testes were narrow and elongated, firmly attached to large lobes of fat tissue, and often lobe-like (Fig. 13a, b). Only primary spermatogonia (Sg 1) were present in the testicular biopsy (Fig. 13c). The testicular wall (TW) was thin, the sperm duct-sinus system (SDSS) was small or absent and the lobules had lumens (Fig. 13 d).

Developing testes

Fishes (n=59) with developing testes ranged from 19.0 to 40.5 mm in FL and 141.6 to 975.3 g in BW and the GW ranged from 1.5 to 3.6 g with a mean GSI value of 0.60 (0.43). The lobes were pinkish to



(a) (b) (c) (d)

Fig. 13. Immature testes of *P. filamentosus*. (a) *In situ* observation; (b) *Ex situ* observation; (c) Testicular biopsy; (d) Histological observation with the presence of Primary spermatogonia (Sg 1), Thin testicular walls (TW) and Sperm duct-sinus system (SDSS)

whitish (Fig. 14a, b) in colour and spermatocytes (Sc) were more clearly visible. Secondary spermatocytes (Sc2), spermatids (St) and spermatozoa (Sz) were uncommon, while primary spermatocytes (Sc1) were more prominent. Histological slides showed broad, long testicles with well-developed sperm crypts and continuous germinal epithelium (CGE) (Fig. 14c, d).

Spawning-capable testes

Fishes (n=98) with spawning capable testes ranged from 31.5 to 76.5 cm in FL and 491.3 to 4346.1 g in BW and the GW ranged from 20.0 to 90.4 with a mean GSI value of 3.49 (1.41). At this stage, the testes were light pinkish or cream colour with solid large lobes (Fig. 15a, b). During this stage, slight pressure on the abdominal region can also release the milt from the testes. Abundant spermatozoa (Sz) in the well-developed sperm duct-sinus system (SDSS) secondary spermatocyte (Sc2) and spermatids (St) were present. Active spermatogenesis occurs at this stage (Fig. 15c, d).

Regressing testes

Fishes (n=42) with regressing testes ranged from 28.0 to 60.5 cm in FL and 378.8 to 3472.3 g in BW and GW ranging between 4.6 and 16.4 g, with a mean GSI value of 1.45 (0.93). Creamish, medium-sized and flaccid testes were observed at this stage (Fig. 16a, b). Spermatocytes (Sc) were widely dispersed around the centre with numerous spermatozoa (Sz) in the fully formed sperm duct-sinus system (SDSS). Residual spermatozoa were also found in the sperm crypts of the regressing testes. A few primary spermatocytes (Sc1) were also observed in the sperm crypts of stained sections of the regressing testes (Fig. 16c, d).

Regenerating testes

Fish (n=35) with regenerating testes were observed within a size range of 37.0 and 51.5 cm; FL, BW from 827.6 to 1961.6 g and

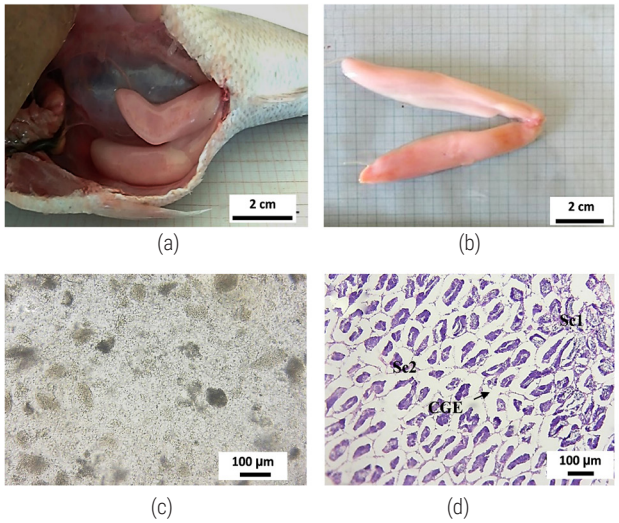


Fig. 14. Developing testes of *P. filamentosus* (a) *In situ* observation; (b) *Ex situ* observation; (c) Testicular biopsy; (d) Histological observation showing the presence of Primary spermatocyte (Sc1), Secondary spermatocyte (Sc2) and Continuous germinal epithelium (CGE)

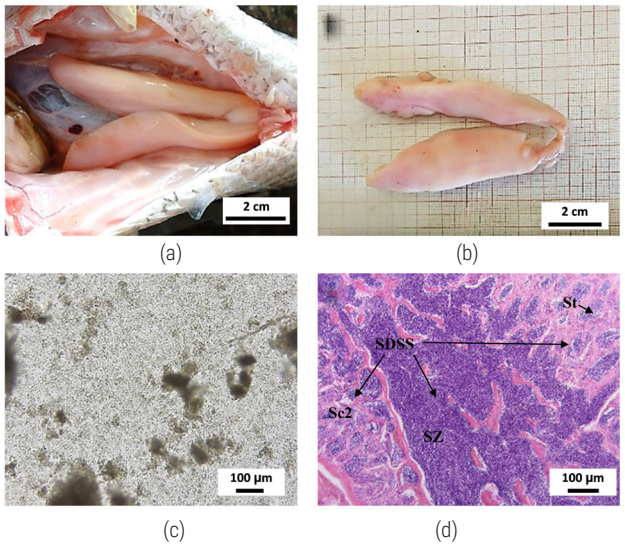


Fig. 15. Spawning capable testes of *P. filamentosus*. (a) *In situ* observation; (b) *Ex situ* observation; (c) Testicular biopsy; (d) Histological observation showing the presence of Sperm duct-sinus system (SDSS), Secondary spermatocytes (Sc2), Spermatids (St) and Spermatozoa (Sz)

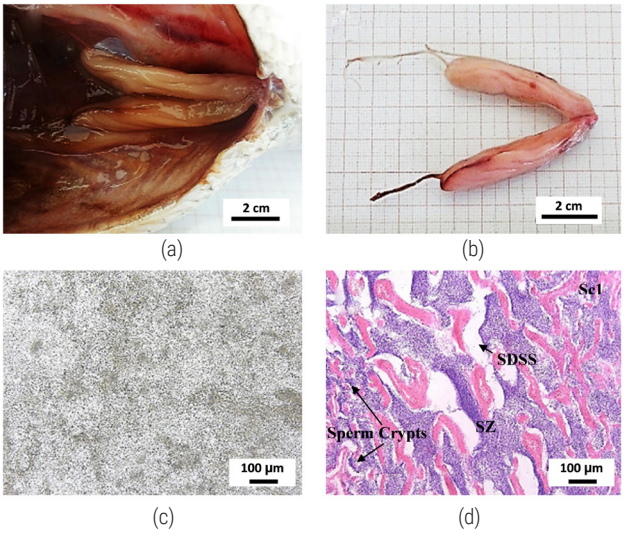


Fig. 16. Regressing testes of *P. filamentosus*. (a) *In situ* observation; (b) *Ex situ* observation; (c) Testicular biopsy; (d) Histological observation showing the presence of Primary spermatocyte (Sc1), Sperm duct-sinus system (SDSS) and Spermatozoa (Sz)

GW ranging from 1 to 4.5 g with a mean GSI value of 0.23 (0.10). Creamish, thin and flaccid testicles (Fig. 17a, b) were observed at this stage. Histologically, it is characterised by the presence of a fully formed sperm duct-sinus system (SDSS) with fewer residual spermatozoa. Primary spermatogonia (Sg1) were widely observed in the stained sections (Fig. 17c, d).

Studies on testicular development in the genus *Pristipomoides* are scanty. In the present study, immature testes with abundant primary spermatogonia (Sg1) and underdeveloped sperm duct-sinus system (SDSS) were observed. Spawning-capable males with abundant spermatozoa within well-developed sperm duct-sinus

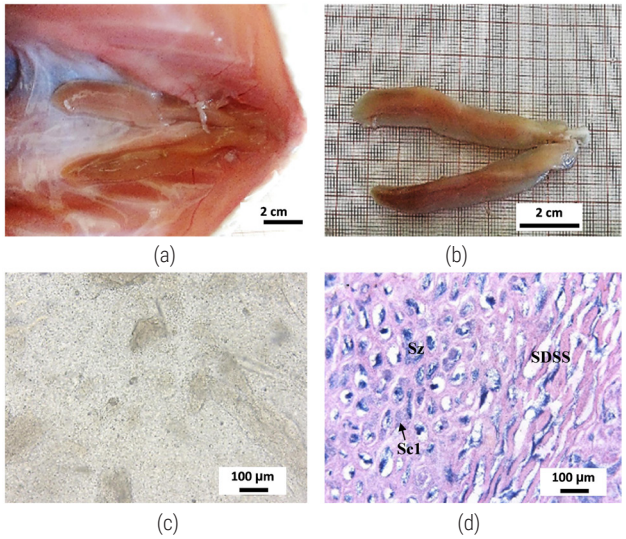


Fig. 17. Regenerating testes of *P. filamentosus*. (a) *In situ* observation; (b) *Ex situ* observation; (c) Testicular biopsy; (d) Histological observation with the presence of Primary spermatocyte (Sc1), Sperm duct-sinus system (SDSS), Spermatozoa (Sz)

systems (SDSS) were observed. Regressing males with very few spermatozoa within empty SDSS were observed. Similar observations were reported by Luers *et al*. (2017) in *P. filamentosus* from the North-western Hawaiian Islands. Uehara *et al*. (2018) described the gonadal development in spawning-capable males of four snapper species from the Okinawan Islands, including *P. filamentosus*.

This is the first comprehensive report on the reproductive biology of *P. filamentosus* from India. The important aspects of the reproductive biology of *P. filamentosus* reported from different parts of the world are summarised in Table 2. The present study mainly focused on biological information such as sex ratio, length at first maturity, fecundity, GSI, maturity phases, oocyte diameter and gonadal development of *P. filamentosus*. A pronounced dominance of females within the fish population was observed. The present study reported elevated GSI values from January to March, indicating higher spawning activity for *P. filamentosus* in this area. Observations on oocyte diameter and gonadal development showed that *P. filamentosus* spawns more than once, annually. *P. filamentosus* is a proposed candidate species for mariculture and the current study will aid in determining the optimal timing for induction of brooders and understanding the basic reproductive traits.

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Table 2. Summary of reproductive studies reported for *P. filamentosus* and the present study

Authority and locality of the study	Sample size	Details of the study	Lm_{50} (cm)	Spawning season	Peak spawning month (s)
Ralston and Miyamoto (1983) / Hawaii	154 Females	Macroscopic examination of maturation phases of ovaries collected during spawning season and the Lm_{50}	FL: 40.0	May-October	August
Kikkawa (1984)/ North-western Hawaiian Islands	150 Females	Studied the distribution of GSI to estimate spawning season spawning conditions and Lm_{50}	FL:42.5	June-December	August
Lokani *et al*. (1990)/ Papua New Guinea	94 Sexes combined	Plotted GSI values on respective lengths to determine the Lm_{50}	FL:34.0	Not reported	Not reported
Mees (1993)/ Mahe Plateau, Seychelles	570 Females; 612 Males	Studied micro and macroscopic examination of ovaries and distribution of GSI to estimate the length at first maturity and spawning season	FL:51.0-53.0	October-April	February and April
Mees and Rossouw (1997)/ Tongan Seamounts Habte (2003)/Eritrea, Red Sea	353 Females 374 Sexes combined	Calculated the minimum length at first maturity Calculated the Lm_{50}.	FL:38.8 TL:53.5	Not reported Not reported	Not indicated Not reported
Luers *et al*. (2017)/ Main Hawaiian Islands	479 Females; 419 Males	Studied spawning seasonality, sex ratio, spawning frequency and sexual maturity and also microscopic examination of histologically prepared gonadal tissues of both sexes	FL: 40.7 F; FL:34.3M	May - September	July
Uehara *et al*. (2018)/Okinawan waters, Japan	176 Females; 144 Males	Studied the sex ratio, Lm_{50}, GSI and spawning seasonality using gonadal histology	FL: 35.7 F; FL: ≥ 20 M	March - October	Not reported
Present study (2023)/ Vizhinjam, Southern coast of Kerala, India	317 Females; 277 Males	Studied sex ratio, Lm_{50}, GSI, fecundity, oocyte diameter and histological analysis of both sexes	FL: 36.6 F FL:35.5 M	January–March	February

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