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# Reproductive biology of fourfinger threadfin, *Eleutheronema tetradactylum* (Polynemidae) from north-eastern Arabian Sea

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## Abstract

The fourfinger threadfin, *Eleutheronema tetradactylum* (Family: Polynemidae) is a commercially important marine finfish species in India. Information on the reproductive biology of this species is limited, which poses challenges for management of its fisheries and exploring its aquaculture potential. Therefore, reproductive biology of *E. tetradactylum* was studied from the north-eastern Arabian Sea based on 824 samples from the coast of Navsari, Gujarat, India during September 2021 to August 2022. *E. tetradactylum* males and females were classified into five reproductive stages: immature, developing, mature, ripe and spent based on macroscopic and histological investigations. Intersex individuals were categorised into three stages *viz.,* initiation of transition, mid-transition and late transition phases. The overall sex ratio of males to females was 1:0.89 ($p<0.05$), indicating a male-dominant population in the region. Length at first sex change in transition fish was 36.2 cm in total length (TL), length at first maturity ($L_{m50}$) of males and females were 24 and 41 cm TL respectively. The absolute fecundity ranged from 1,58,080-12,53,571 eggs, with mean value 5,85,886±53,315.1. Both males and females showed seasonal variations in the GSI values, with the highest values observed from May to October with a peak in August. The presence of all stages of oocytes in mature fish indicated that *E. tetradactylum* is a multiple spawner with asynchronous ovary having prolonged spawning season from May to November with a peak during August to September that coincides with the monsoon season in north-eastern Arabian Sea.

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## Introduction

The fourfinger threadfin, *Eleutheronema tetradactylum* (Family: Polynemidae), is a commercially important marine finfish species which has high demand in domestic and export markets for its excellent meat quality. This species is a euryhaline fish commonly found in shallow coastal waters, estuaries and rivers (Motomura *et al.,* 2002; Pember *et al.,* 2005) and is distributed in tropical and subtropical regions of the Indian and western Pacific oceans (Motomura, 2004).

In India, *E. tetradactylum* forms a minor fishery on the east coast and the north-west coast (Kagwade, 1970) and is caught in diverse gears including gill nets, bag nets, shore seines and trawl nets (Pillay and Ghosh, 1962; Kagwade, 1968). *E. tetradactylum* is a sequential protandrous hermaphrodite (Pember *et al.,* 2005; Shihab *et al.,* 2017). It is carnivorous in nature, preying on crustaceans and small fishes with occasional cannibalism (Hena *et al.,* 2011). Juveniles of *E. tetradactylum* inhabit estuaries and adults are found in shallow muddy coastal waters (Motomura *et al.,* 2002, 2015). The population of *E. tetradactylum* is declining in tropical and subtropical coastal regions as a result of overfishing and pollution (Motomura *et al.,* 2002; Newman *et al.*, 2011). It requires effective localised fisheries management for its long-term sustainability (Qu *et al.,* 2020).

Studies on the reproductive biology of *E. tetradactylum* in different regions of the world suggest that the species is gonochoristic in India (Patnaik, 1969; Gopalakrishnan, 1972) and Singapore (Chao *et al.,* 1994). In eastern Australia, *E. tetradactylum* is reported to be protandrous (Stanger, 1974; Russel, 1988; McPherson, 1997). Pember *et al.* (2005) reported that *E. tetradactylum* is a protandrous hermaphrodite in Australian waters that attains sexual maturity as male at the end of the first year of life at a length of 20 cm and later changes to female at 40 cm. Zamidi *et al*. (2012) studied fecundity and reproductive seasonality of *E. tetradactylum* in Malaysia. Soe *et al.* (2023) studied reproductive aspects of *E. tetradactylum* from Thailand waters. Contrary to earlier reports of *E. tetradactylum* gonochorism in Indian coastal waters (Patnaik, 1969; Goplakrishnan, 1972). Shihab *et al.* (2017) reported protoandrous hermaphroditism in the species based on histological studies.

Previous studies on *E. tetradactylum* in India, primarily during 1940s to 1970s, report conflicting spawning seasons in Bombay waters and Chilka Lake. Bal and Pradhan (1946) suggested year-round spawning, while others noted peak breeding seasons from January-April and July-September (Karandikar and Palekar, 1950), January-June (Jhingran and Natarajan, 1966; Patnaik, 1970; Kowtal, 1972). Since then, significant changes have occurred in the environment such as habitat degradation, climate change and increased fishing pressure.

The present study aimed to investigate the reproductive biology of *E. tetradactylum* in the north-eastern Arabian Sea, India. The outcomes of this study will be helpful in management of its fisheries in the region and also provide baseline data for the development of captive broodstock of *E. tetradactylum* for breeding and seed production for conservation and aquaculture purposes.

## Materials and methods

### Gonad sampling

A total of 824 specimens of *E. tetradactylum* ranging from 12 to 61.5 cm in total length (TL) were collected from artisanal and commercial gillnet landings at Dholai Port, Billimora Taluka, Navsari District, Gujarat, India, from September 2021 to August 2022. The Dholai Port (20°44’19”N; 72°53’42”E) is located 37.5 km from Navsari. The collected specimens were kept in ice boxes and transported to the laboratory of Navsari Gujarat Research Centre of ICAR-Central Instiitute Brackishwater Aquaculture (ICAR-CIBA). The total length (TL) of each fish was measured to the nearest 1 mm using a measuring scale, while, the body weight (TW) of each fish was measured to the nearest 0.1 g with a digital balance. The weight of gonads of each individual was recorded to the nearest 0.01 g.

### Macroscopic classification of sexes and maturity stages

The individual specimens were dissected, and gonads were taken out from the body cavity, and examined macroscopically to identify male, intersex and female. Male and female individuals were classified into five stages, based on macroscopic examination.

Following the method of Kume *et al.* (2006) males were classified as (i) Immature, (ii) Developing, (iii) Maturing, (iv) Ripe and (v) Spent. Intersex individuals were classofied into 3 stages following the method of Pember *et al.* (2005) into, (i) Early transition phase; (ii) Mid transition phase and (iii) Late transition phase. Females were classified following the method of Afonso-Dias *et al.* (2005) into, (i) Immature, (ii) Developing, (iii) Maturing (iv) Ripe and (v) Spent. The reproductive cycle was described by analysing the variation in the monthly composition of different maturity stages.

### Histological examination of gonads

Gonads from males, intersex individuals and females were fixed in 10% neutral buffered formalin (NBF). Three sub-samples were collected from the anterior, middle and posterior region of both gonadal lobes and processed for histological analysis. All tissues were thoroughly washed in tap water for 6-8 h before being loaded into the automatic tissue processor. Tissue samples were dehydrated in an ascending series of isopropyl alcohol solution (70 to 90%) and xylene; and were finally embedded in paraffin wax. The embedded tissues were molded into blocks and sectioned to achieve 3-5 µm transverse sections using a microtome (Leica RM2125 RTS). The sections were stained with Harris haematoxylin and eosin and then mounted with DPX (Roberts, 1989). A total of sixty-five gonads were processed for histological examination. The slides of different maturity stages were examined under Zeiss Axio Scope A1 microscope and photomicrographs were taken with Jenoptik ProgRes C3 digital camera attached to the microscope. Microscopic characterisation of gonads was conducted following the histological criteria described by Brown-Peterson *et al*. (2011).

### Sex ratio

The sex ratio was determined by dividing the total number of females by the total number of males. Subsequently, a chi-square ($\chi^2$) test was performed to evaluate the sex ratio between months.

### Length at first sex change

Length at first sex change (the size at which 50% of the population are females) was estimated by applying logistic regression to the proportion of females across the length classes of both male and female populations using the logistic equation (Soe *et al.,* 2023):

$$Pls = (1 + \exp^{-\ln 19(S-S50)/S95-S50)})^{-1}$$

where, Pls is the proportion of male and female in each class interval S. S50 and S95 refer to the lengths at which 50 and 95% of the population are females, respectively.

### Length at first maturity

Length at first maturity was determined from the logistic equation (King, 2007). P= 1/ {1+ exp [-r x (TL - $L_m$)]} where P is the proportion of mature individuals in a length class, TL is total length, r is intercept and $L_m$ is slope. The r and $L_{m50}$ were estimated using the non-linear regression solver from the ratio of reproductive to non-reproductive fish groups.

## Gonadosomatic index

To estimate changes in the gonad weight during the annual reproductive cycle and spawning season, monthly gonadosomatic index (IG) (De Vlaming *et al.,* 1981) was calculated using the formula:

IG = Gonad weight (g)/ Bodyweight (g) ×100

## Fecundity

Fecundity was determined using the gravimetric method (Hunter, 1992). Sub sample of 0.05 g was taken from the anterior, middle and posterior portions of the fish ovary, and the number of eggs were counted. The fecundity (F) was determined using the equation, F = SS/WS × W0 where, SS = Number of oocytes in sub samples; WS = weight of sub sample and WO = weight of ovary. Relative fecundity was estimated by dividing total fecundity with the body weight of fish. The relationship between fecundity and total body length, fish body weight and ovary weight were estimated by linear regression analysis.

# Results

## Macroscopic and histological characteristics of gonadal development stages in male *E. tetradactylum*

## Immature

The testes are minute, whitish strand or ribbon-like structures (Fig. 1a). Histological examination revealed the dominance of connective tissues with nests of spermatogonia. Immature testes lack lumen (Fig. 2a). The proliferation of spermatogonia occurs through mitotic division. Immature males were observed from January to May with a peak during February to March (Fig. 7a).

## Developing

Testes are moderately enlarged, whitish and narrower towards the posterior end. They occupy about 30-40% of the length of the body cavity (Fig. 1b). Initiation of spermatogenesis begins at this stage. Developing phase of testes revealed presence of spermatocytes undergoing active spermatogenesis, development of lumen within the lobule and lack spermatozoa (Fig. 2b). Developing stage of testes was observed during December to July, with a peak in April (Fig. 7a).

## Maturing

Testes enlarged with milt, occupying more than half of the body cavity. No free running milt oozes on pressure (Fig. 1c). Mature phase of testes contains spermatocytes in all the stages of spermatogensis. Germinal epithelium is discontinuous throughout the testes (Fig. 2c). Mature testes were observed throughout the year with a peak during May and June, with limited numbers observed during December to February (Fig. 7a).

## Ripe

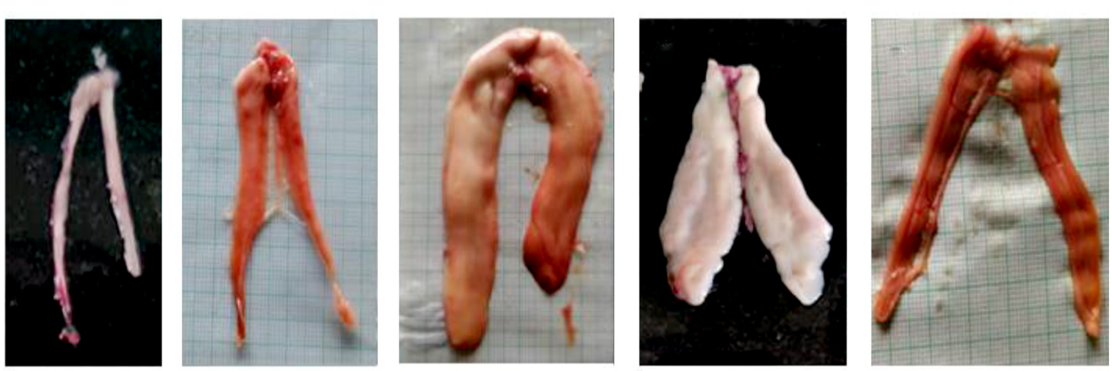
The testes are swollen and milky white in appearance (Fig. 1d). When gentle pressure is applied to the abdomen, milt is released freely. Ripe stage of testes consists of all the stages of spermatogenesis. Germinal epithelium is discontinuous throughout the testis. Seminiferous tubules are filled with spermatozoa in the lumen of the lobules and ducts (Fig. 2d). Running males were observed from May to November, with a peak in August-September (Fig. 7a).

## Spent and recovering

The testes are flaccid and shrunken, and reddish brown in appearance with residual milt (Fig. 1e). Histological examination of spent testes revealed connective tissues with presence of residual spermatozoa in the lumen of the lobe (Fig. 2e). Spent stage of testis was observed from June to January, with a peak during November-December (Fig. 7a).

## Macroscopic and histological classification of gonads in transitional individuals of *E. tetradactylum*

Based on macroscopic and histological examination of gonads, transitional individuals of *E. tetradactylum* were classified into three stages, (i) Early transition phase; (ii) Mid transition phase and (iii) Late transition phase.



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

Fig. 1. Macroscopic observations of different maturity stages of *E. tetradactylum* testes. (a) Immature; (b) Developing; (c) Maturing; (d) Ripe; (e) Spent

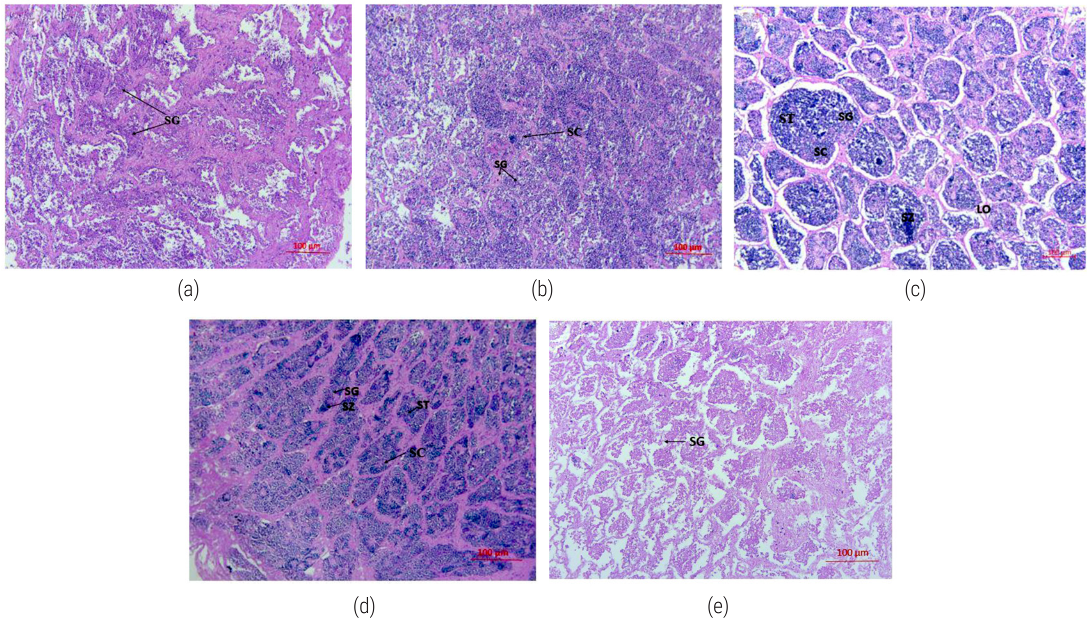



Fig. 2. Histo-micrograph of different maturity stages of *E. tetradactylum* testes. (a) Immature; (b) Developing; (c) Maturing; (d) Ripe; (e) Spent. SG: Spermatgonium; SC: Spermatocytes; ST: Spermatids; SZ: Spermatozoa

## Early transition

In the early stages of transition in protandrous fish, the gonads are white and resemble typical male gonads, retaining the characteristics of typical testes (Fig. 3a). Gonads were characterised macroscopically by the presence of dominant whitish testes with milt which oozes upon pressure on the abdomen. The ventral outer region of the gonads had noticeable pinkish orange layer of ovarian tissue, surrounded by testicular tissue which was located along the inner dorsal region of the gonad. The microscopic section of early transition phase revealed that, testicular regions had elongated lobules containing crypts with dispersed spermatogonial cells. The tubules and internal lumen were predominantly occupied with advanced spermatogenesis stages (Fig. 4a). The ovarian region consists of early stages of oocytes, chromatin nucleolar and perinucleolar stages (Fig. 4a). The early transition stage of *E. tetradactylum* was observed throughout the year, except during August and September (Fig. 7b).

## Mid transition phase

Mid transition phase of bisexual gonads was characterised by the presence of orange translucent layer of ovarian tissues within testicular region (Fig 3b). The testes contain milt which oozes out

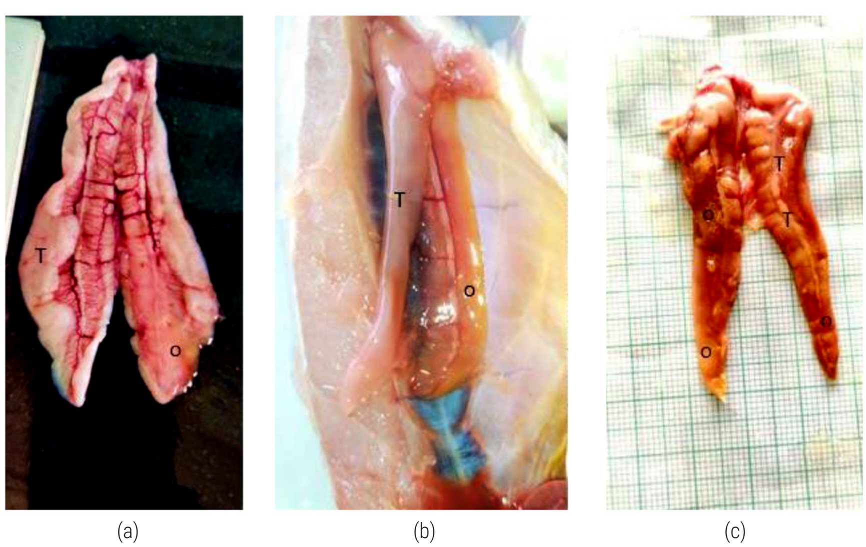



Fig. 3. Macroscopic observations of different stages of transitional gonads of *E. tetradactylum*. (a) Early transistion; (b) Mid transition; (c) Late transition. T: Testis; O: Ovary

with pressure on abdominal region. Morphologically, the ovarian region of bisexual gonads appears similar as immature ovary. The percentage of ovarian tissue over testicular tissue ranged from 40 to 50% and it varied with the sampled individuals. Histological sections revealed the components of testicular and ovarian regions demarcated by the presence of connective tissues. The ovarian region consists of early developing oocytes (perinucleolar stage and chromatin nucleolar stages). The testicular region is dominated by spermiogenesis stage of spermatocytes and spermatozoa (Fig 4b). It appears as advance mature stage of testes. The mid transition stage of *E. tetradactylum* was observed throughout the year except during August and September, with a peak in March (Fig. 7b).

## Late transition phase

The gonads in the late transition phase are characterised by a dominance of ovarian tissue, which ranges in colour from light to dark orange (Fig. 3c). There is a notable reduction in testicular tissue, with only a thin, whitish layer remaining on the inner dorsal surface of the gonad. Histological cross sections revealed the presence of perinucleolar and chromatin nucleolar stages of oocytes. The testicular region was dominated by early stages of spermatogonia and spermatocytes and empty lumen with residual spermatids and spermatozoa (Fig. 4c). Further, some components of ovarian region had residual testicular tissue showing signs of degeneration. The late transition stage was observed from October to May, with a peak in February and April. Absence of late transition individuals was observed from June to September (Fig. 7b).

## Macroscopic and histological characteristics of ovarian development stages in female *E. tetradactylum*

Based on macroscopic and histological observations, the ovary of *E. teradactylum* was classified into five development stages *viz.*, (I) Immature, (ii) Developing, (iii) Mature, (iv) Ripe and (v)Spent.

## Immature

The immature ovaries of *E. tetradactylum* appeared as small, orange cylindrical lobes (5-8 mm wide), occupying 15-20% of the body cavity (Fig. 5a). No oocytes were visible to the naked eye. Histological observation indicated the presence of early perinucleolar oocytes and few late perinucleolar oocytes. Wall of the ovary was very thin with little space between the oocytes (Fig 6a). Oil droplets and muscle bundles absent. Immature *E. tetradactylum* were primarily recorded in January (70.6%) and February (56.5%), with moderate occurrence in March (37.1%) and April (26.3%) and were absent from June to December (Fig. 7c).

## Developing

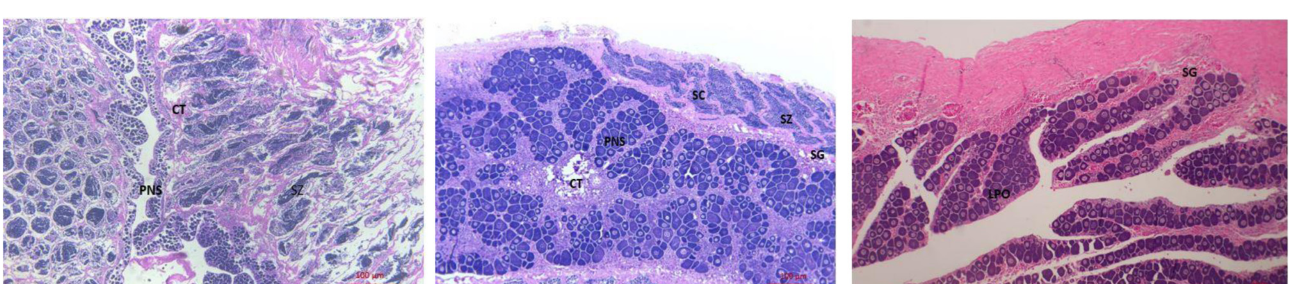
The ovary was cylindrical in shape with folds and was slightly larger than stage 1, occupying 25% of the body cavity (Fig. 5b). Histological sections revealed the dominance of late stages of perinucleolar oocytes and cortical alveolar oocytes. Accumulation of yolk globules with a few oil droplets was seen in the cytoplasm, with the chorion layers becoming clearly visible (Fig. 6b). The prevalence of developing individuals was highest from February to May, peaking in February (43.5%) and March (45.7%) (Fig. 7c).

## Maturing

The mature ovaries were larger in size and yellowish in colour, and occupied about 85-90% of the body cavity. Granular oocytes were visible from the ovarian wall (Fig. 5c). Histological sections of maturing ovary revealed large numbers of early vitellogenic oocytes (Vt1) with centrally located germinal vesicle and a few numbers of perinucleolar oocytes and cortical alveolar oocytes (Fig. 6c). As maturation progressed, the size of the oocytes increased and the oil droplets grew larger. The chorion began to separate into two distinct layers, the inner and outer zona radiata. Maturing fish were observed from March to November, with higher occurrence during the monsoon (June-August) (Fig. 7c).

## Ripe

Fully swollen ovaries occupied the entire body cavity, appearing yellowish-orange with visible blood vessels (Fig. 5d). Hydrated oocytes were free-flowing under slight pressure to the abdomen. Histological observations revealed the presence of advanced vitellogenic oocytes with yolk granules in the cytoplasm. In ripe oocytes, the number of oil droplets increased and merged to form a single oil globule (OG), which gradually pushed the germinal vesicle to the periphery of the cytoplasm (Fig 6d). The yolk globules also combined to create fluid yolk (FY). A significant percentage of oocytes exhibited complete germinal vesicle migration (GVM) and germinal vesicle breakdown (GVBD). Ripe *E. tetradactylum* females were predominantly observed from June to October, with peaks in August (44.4%) and September (45%). This stage was absent during March to December (Fig. 7c).




(a) (b) (c)

Fig. 4. Histo-micrograph of different stages of transitional gonads of *E. tetradactylum. (*a) Early transition; (b) Mid transition; (c) Late transition. PNS: Perinucleolar oocyte; CT: Connective tissue; SC: Spermatocytes; SG: Spermatogonium; SZ: Spermatozoa

## Spent

Ovaries were shrunken, dark red to brown in colour and highly vascularised with a few ruptured blood vessels. They continued to shrink in size and occupied half the length of the body cavity (Fig. 5e). Histology of the spent ovary revealed empty spaces and the presence of post-ovulatory follicle complexes, chromatin nucleolar and perinucleolar stages, and cortical alveolar oocytes (Fig. 6e). Spent stage of *E. tetradactylum* was observed during July to January, with a peak during November and December. No spent females were observed during February to June (Fig. 7c).

## Sex ratio

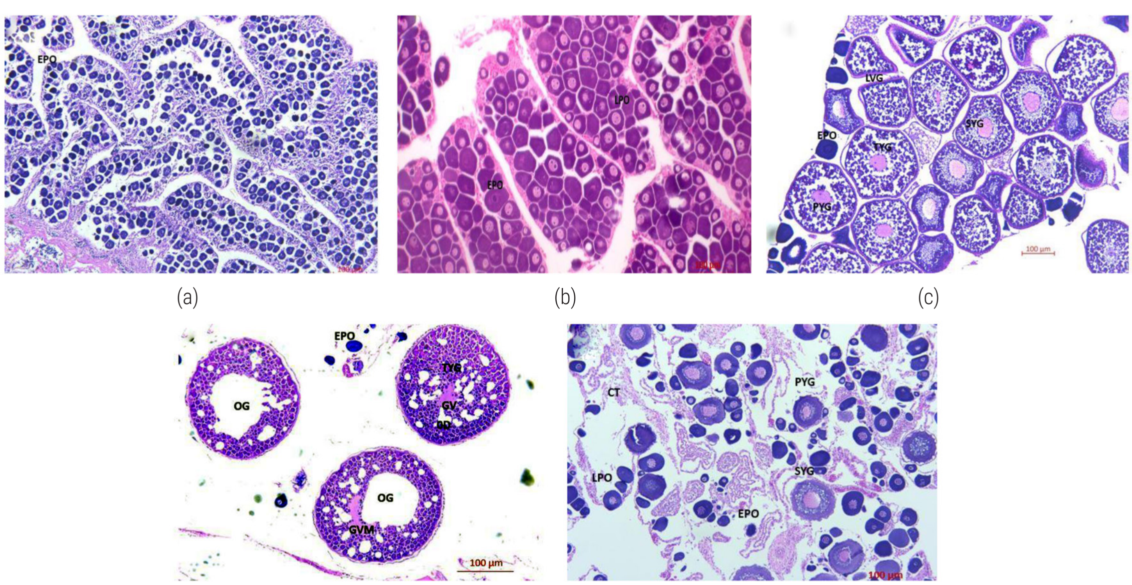
Out of the 824 sampled individuals, 371 (45.02%) were males, 333 (40.41%) were females and 120 (14.56%) were transitional. The average measures of length and weight of males, females and transition individuals were 28.87±0.365 cm TL and 329.42±10.23 g BW, 44.71±0.258 cm TL and 818.0±15.93 g BW and 36.44±0.25 cm TL and 438.13±10.56 g BW, respectively. The overall sex ratio of males to females was 1:0.9 ($\chi^2$ = 14.16, p<0.005) (Table 1). The population was slightly more male-dominated. November, December and January months showed significant dominance by males compared to female (p<0.05), whereas June, July, August and September showed more or less equal proportions of males and females (Table 1).

## Length at first sex change

The length at first sex change was estimated at 36.2 cm (Fig. 8). The smallest transitional fish measured 29.5 cm and weighed 310 g. The largest was 45 cm and weighed 759 g. Majority of the transitional fish was observed in the size range of 34 to 39 cm.

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

Fig 5. Macroscopic observations of different maturity stages of *E. tetradactylum* ovary. (a) Immature; (b) Developing; (c) Maturing; (d) Ripe; (e) Spent




(a) (b) (c)

(d) (e)

Fig 6. Histo-micrograph of different maturity stages of *E. tetradactylum* ovary. (a) Immature; (b) Developing; (c) Maturing; (d) Ripe; (e) Spent EPO: Perinucleolar oocytes; LPO: Late perinucleolar oocyte; PYG: Primary yolk granule; SYG: Secondary yolk granule, TYG: Tertiary yolk granule; LVS: Lipd vesicle stage; OD: Oildroplets, OG: Single oil globule; GV: Germinal vesicle; GVM: Germinal vesicle migration

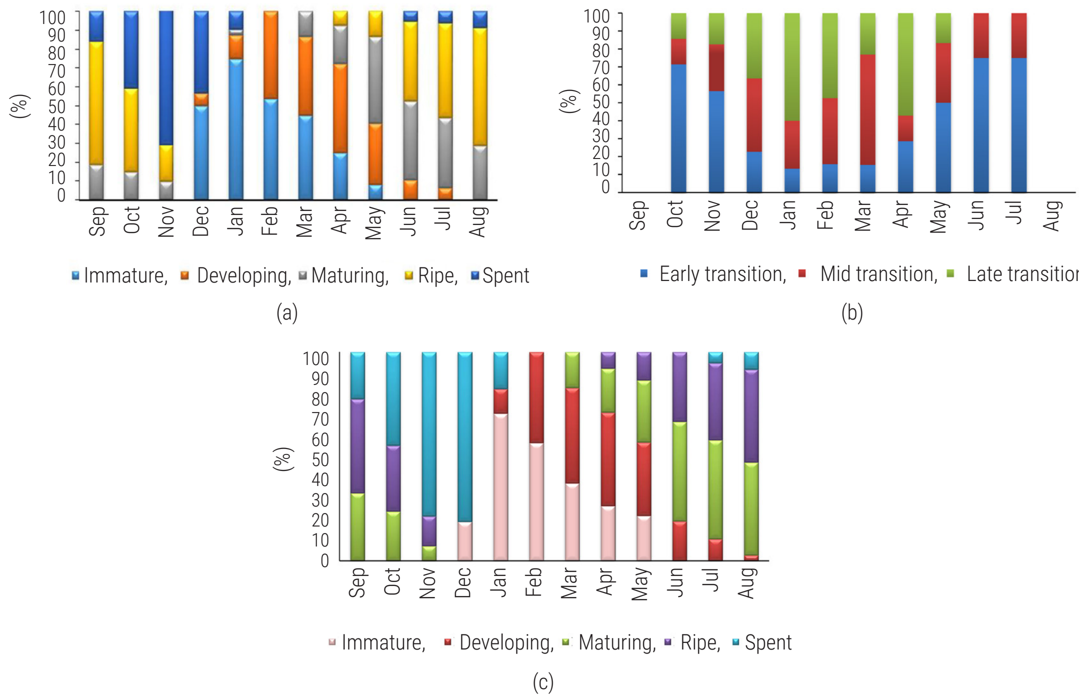



Fig. 7. Monthly distrubution of maturity stages of *E. tetradactylum* from September 2021 to August 2022. (a) Male; (b) Transitional; (c) Female

Table 1. Monthly sex ratio of *E. tetradactylum*

| Months | Total | Number of males | Number of females | Male :Female ratio | Chi-Square ($\chi^2$) | p value |
|---|---|---|---|---|---|---|
| January | 49 | 32 | 17 | 0.53 | 4.591837 | 0.032125 |
| February | 51 | 28 | 23 | 0.82 | 0.490196 | 0.48384 |
| March | 73 | 38 | 35 | 0.92 | 0.123288 | 0.725496 |
| April | 78 | 40 | 38 | 0.95 | 0.051282 | 0.820847 |
| May | 73 | 37 | 36 | 0.97 | 0.013699 | 0.906827 |
| June | 40 | 19 | 21 | 1.11 | 0.1 | 0.75183 |
| July | 35 | 16 | 19 | 1.19 | 0.257143 | 0.61209 |
| August | 71 | 35 | 36 | 1.03 | 0.014085 | 0.90553 |
| September | 78 | 38 | 40 | 1.05 | 0.051282 | 0.820847 |
| October | 65 | 27 | 38 | 1.41 | 1.861538 | 0.172447 |
| November | 45 | 31 | 14 | 0.45 | 6.422222 | 0.01127 |
| December | 46 | 30 | 16 | 0.53 | 4.26087 | 0.039 |
| Total | 704 | 371 | 333 | 0.9 | 2.051136 | 0.152093 |

$p \leq 0.05$

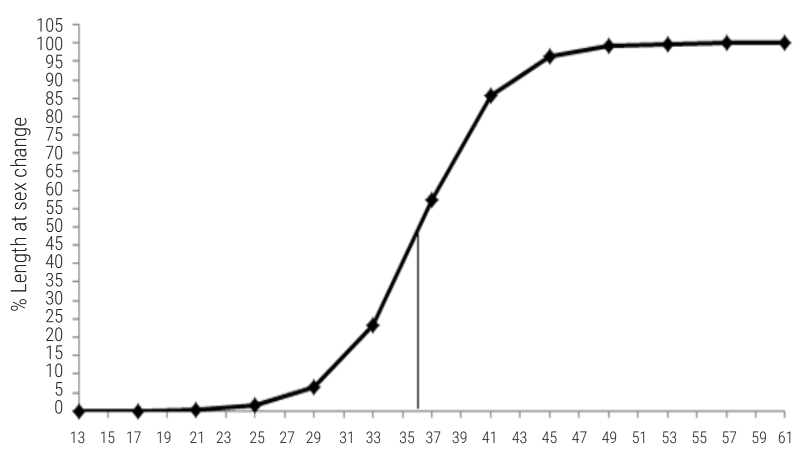



Fig. 8. Length at first sex change of *E. tetradactylum* from Gujarat coast

## Length at first maturity

The length at first maturity of male and female *E tetradactylum* were estimated at 24 and 41 cm TL respectively (Fig 9a and b). The smallest male observed with ripe gonads measured 23 cm TL and 180 g BW, while the largest male recorded measured 57.5 cm TL and 1750 g BW. The smallest and largest female observed with mature gonads measured 37 cm TL and 490 g BW and 61.5 cm TL and 1950 g BW respectively.

## Gonado-somatic index (GSI)

The monthly mean GSI of *E. tetradactylum* showed seasonal variations (Fig. 10). In males, GSI was lowest in January (0.15±0.07), peaked in August (1.123±0.03) and declined from November. Females had the lowest GSI from December to February (1.23±0.08) and peaked in August (9.09±0.45). Transitional individuals followed a similar trend as males, with a peak in August (1.07±0.09).

The reproductive cycle of *E. tetradactylum* off Gujarat coast includes five maturity stages. Immature and developing stages (I and II) dominated from January to March. Late maturing gonads marked the pre-spawning phase (April-June). Ripe males and females were prevalent from May to October, with peak spawning in August. Spawning ceased from October to December, indicated by spent fish with post-ovulatory follicles, chromatin nucleolar and perinucleolar stages.

## Fecundity

The fecundity of *E. tetradactylum* was analysed using 30 mature fish with total lengths ranging from 38 cm to 61.5 cm and body weights from 550 to 2150 g. The fecundity was found to vary from 1,58,080 to 12,53,571 eggs averaging 5,85,886±53315.1. The relative fecundity ranged from 287.41 to 886.364 eggs per g body weight with a mean value of 593.229. Fecundity showed a positive logarithmic relationship with increasing total length, body weight, and gonad weight of the fish (Fig.11a and b).

# Discussion

The present study provides detailed information on the reproductive pattern of *E. tetradactylum* from north-eastern Arabian Sea India. Eight gonadal stages have been documented in male and female *E. tetradactylum* in previous studies (Pember *et al.,* 2005; Shihab *et al.,* 2017; Soe *et al.,* 2023); three transitional stages were described by Pember *et al.* (2005) and two stages by Shihab *et al.* (2017) and Soe *et al*. (2023). In the present study, through macroscopic and microscopic examination, five distinct maturation stages were delineated in male and female *E. tetradactylum* along with three stages in transitional individuals. The study indicated that males show asynchronous readiness and spawn multiple times, as the ripe stage shows peaks during September, June, July and August, with notably high percentages of mature males in June

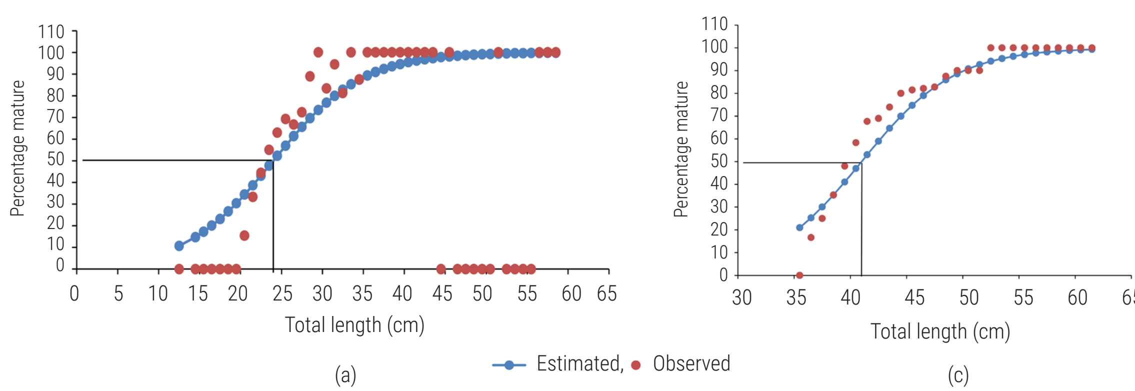



Fig. 9. Length at first maturity of *E. tetradactylum* from Gujarat coast. (a) Male; (b) Female

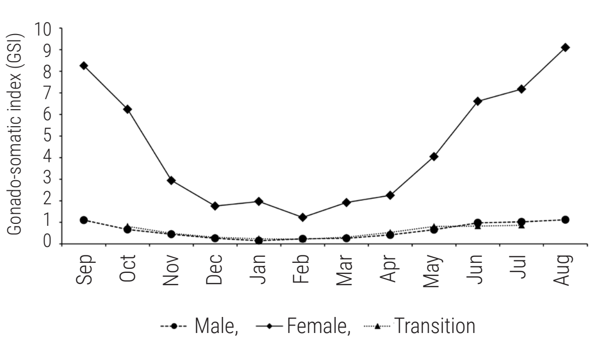



Fig. 10. Monthly mean GSI of male and female *E. tetradactylum* from Gujarat coast

(42.1%) and July (50%). This indicates that members of the male population attain spawning readiness at different intervals, rather than all individuals maturing simultaneously. Further, microscopic observation of testes revealed the presence of different stages of spermatogenesis, indicating the asynchronous pattern of spawning. Similarly, the presence of ripe and mature females from April to November, indicates asynchronous oocyte development. Ogino *et al.* (2023) stated that *E. tetradactylum* is an asynchronous spawner, as evidenced by its year-round hatching and significant variations in reproductive timing among individuals. Soe *et al.* (2023) described asynchronous ovarian development and synchronous testis development in the same species in Thai waters.

*E. tetradactylum* is recognised as a sequential protandrous hermaphrodite in Australian waters (Pember *et al.,* 2005), as well

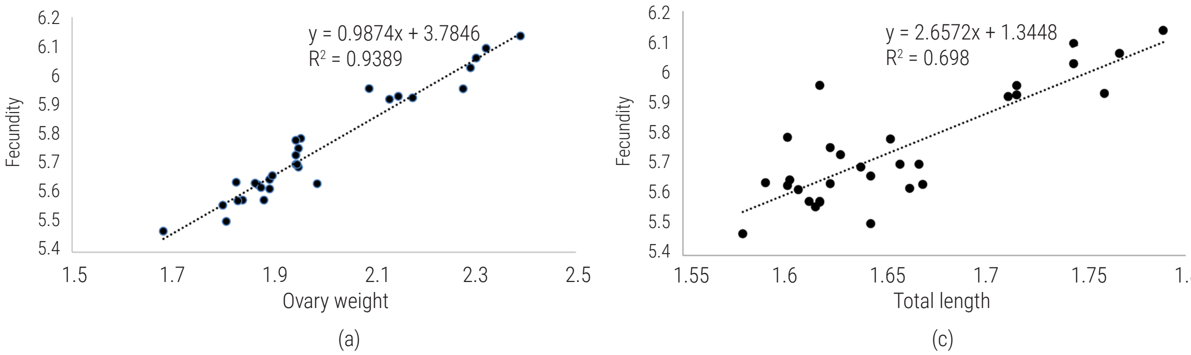



Fig. 11. (a) Relationship of fecundity with (a) Total length; (b) Ovary weight

as in India (Shihab *et al.,* 2017) and Thailand (Soe *et al.,* 2023). Based on macroscopic and microscopic examination of the gonads of intersex individuals of *E. tetradactylum*, they were classified into three stages *viz.,* early transition, mid-transition and late transition phases. In sequential hermaphrodites, the transition from one sex to another sex usually involves the degeneration of the initial sex's germinal tissue, followed by the development of the subsequent sex's tissue (Shapiro, 1981). In cases with delimited gonads, the tissue from the initial sex often shrinks significantly during the transition and disappears completely, leaving no evidence of the previous sex (Sadovy and Shapiro, 1987). In *E. tetradactylum*, male and female tissues are separated by a connective tissue membrane, creating a delimited structure (Shihab *et al*., 2017). In the present study, intersex individuals were observed in the size range of 29.5 to 43 cm total length. The highest percentage of intersex *E. tetradactylum* was observed during November and December immediately following the spawning season. This shows that sex change in *E. tetradactylum* is size-dependent, with mature males reaching a threshold length and then transitioning to female after spawning. In line with our observations, Pember *et al.* (2005) reported that approximately 43% of *E. tetradactylum* individuals in Australian waters had completed sex change and matured as females by the second spawning period. This pattern suggests that sex change in *E. tetradactylum* is probably initiated upon attaining a critical body length.

In the present study from the coastal waters of Gujarat, the sex ratio of *E. tetradactylum* observed was 1:0.89. *E. tetradactylum* is a protandrous hermaphrodite fish species and there was significant dominance of males during November, December and January months. Protandrous fishes typically exhibit bimodal frequency distributions. Generally males being smaller in size and more abundant in the population, sex ratio is often skewed toward males (Kawaguchi and Marumo, 1967; Moyer and Nakazono, 1978; Fricke, 1979, Moore, 1979). Similarly, Soe *et al.* (2023) reported male to female sex ratio of 1:0.69, with males being dominant throughout the year in *E. tetradactylum* from the Thailand coastal waters. Zamidi *et al.* (2012) reported dominance of males over females in Malaysian waters. Nikolsky (1980) suggests that in natural population, ideal sex ratio is close 1:1. However, several factors influence variations in sex ratio such as fishing activity, mortality, occupancy of different habitats by sexes (Vincentini and Araujo, 2003; Lewis and Fontoura, 2005; Lauer *et al.,* 2008).

Earlier reports from Indian waters suggested that *E. tetradactylum* was gonochoristic (Patnaik, 1969; Goplakrishnan, 1972). However, there are several reports that confirmed *E. tetradactylum* to be a protandrous hermaphrodite (Pember *et al.,* 2005; Shihab *et al.,* 2017; Soe *et al.,* 2023). There are no detailed reports available for length at first sex change of *E. teradactylum* in Indian waters. The present study indicated that the length at first sex change was 36.2 cm on the Gujarat coast of India. Similarly, Pember *et al.* (2005) reported sex change at 39.7 cm in *E. tetradactylum* from Queensland of Australia. Soe *et al.* (2023) reported sex change at 27.58 cm total length and 419.39 g body weight. Shihab *et al.* (2017) found that transition to female started at 28.0 cm. In the present study, the smallest intersex fish measured 29.5 cm and weighed 310 g. The largest was 45 cm and weighed 759 g. Several factors influence sequential hermaphroditism like social interaction between the sexes, body size, sex ratio, food and niche availability (Devlin and Nagahama, 2002; Avise and Mank, 2009). In the present study, a total of 14.54% of transitional fish was observed in a year with the predominance during December-February after the spawning season. Lowest proportion of intersex fish were observed during peak breeding season, which may be due to completion of transition phase. Kagwade (1968) reported that the occurrence of intersex fish of *Polynemus heptadactylus* in monthly samples varied from 2 to 45% depending on the time of year. Several studies have found a higher proportion of intersex fish after the reproductive period (Fujii, 1971; Moore, 1979). Stanger (1974) suggested that sex change in *E. teradactylum* initiated immediately after spawning season in eastern Queensland, Australia and transition took a minimum of one year.

Length at first maturity ($L_{50}$) is an important biological index for the management and conservation of fish populations (Soares *et al.,* 2020). Sexual dimorphism exists in *E. tetradactylum*; females are larger than males. It is a protandrous hermaphrodite, initially maturing as male and later changing sex to female (Pember *et al.,* 2005). In the present study, the size at first maturity *of E. tetradactylum* was estimated to be 24 cm for males and 41 cm for females. Pember *et al.* (2005) reported that male and female lengths at maturity were 20.1 and 39.7 cm respectively, from Australian waters. Soe *et al.* (2023) reported that length at first maturity for male and female from Samut Prakan Province of Thailand was 25.78 and 31.40 cm respectively. Tirtadanu *et al.* (2018) estimated 39.6 cm length at first maturity from pooled sex from Indonesia. Minor variations in length at first maturity in different regions are attributed to prevailing biotic and abiotic factors (Teixeira *et al.,* 2010; Ugrin *et al.,* 2023). Early research in India on reproductive biology of *E. tetradactylum* indicate that the species is gonochoristic (Patnaik, 1969; Goplakrishnan, 1972). Karna *et al.* (2012) estimated the length at first maturity of *E. tetradactylum* at 31.5 cm using pooled sex data (n = 69) from Chilka Lagoon of India. Shihab *et al.* (2017) reported protandrous hermaphroditism in *E. tetradactylum* through histological analysis. The present study provides information on the length at sex change and first maturity of *E. tetradactylum* from the Gujarat coast of India. This data is useful for mesh size regulation and fisheries management in the region, as well as providing baseline data for broodstock development in captivity for artificial propagation of *E. tetradactylum*.

Fecundity is a crucial biological parameter that influences the assessment of commercial potential of fish stocks (Gomez-Marquez, 2003). The breeding success of fish species is directly dependent on understanding their fecundity (Bromage *et al.,* 1990). In our study, the fecundity of *E. tetradactylum* ranged from 158080 to 1253571 with mean value of 585886±53315.1 and had a positive correlation with total length and body weight which is in agreement with the previous studies by Zamidi *et al.* (2012) who reported *E. tetradactylum* is a highly fecund species in Malaysian waters with 3,41,358 to 1114757 eggs. Nesrul *et al.* (2014) reported $10.05 \times 10^5$ to $20.91 \times 10^5$ eggs from Bay of Bengal. Abdul Samad (1987) reported 1,79,546 to 4,88,927 from Musi Estuary, Indonesia. Soe *et al.* (2023) reported fecundity of 1,85,000 from Thailand waters. Fecundity varies annually, and is directly proportional to the size, age and condition of the fish (Horwood *et al.,* 1986; Rijnsdorp, 1991; Kjesbu *et al.,* 1998). Egg production varies among individuals of the same size and age (Mathur and Ramsey, 1974; Emery and Brown, 1978). Fecundity is also influenced by the quantity and quality of food, as well as several environmental factors (Taylan *et al.,* 2017).

The gonadosomatic index is widely used to improve accuracy in determining the maturity stages, reproductive time and spawning season (Lowerre–Barbieri *et al.,* 2011). In the present study, the highest GSI was observed from May to October, indicating that the fish spawns during the pre-monsoon to post-monsoon with peak spawning activity during the monsoon season. Further, the minimum GSI was recorded during the winter seasons from December to February. Similarly, Karandikar and Palekar (1950), Sarojini and Malhotra (1952) and Patnaik (1970) reported the presence of mature ovaries in *E. tetradactylum* over prolonged periods of six months or more in Indian waters with two peak breeding seasons - March to April and July to August. Nesarul *et al.* (2014) reported two peak breeding seasons of *E. tetradactylum* along Bangladesh coast, one from February to March and another from July to August. Soe *et al.* (2023) reported year-round multiple spawning behaviour of *E. tetradactylum* in Thailand waters with a peak breeding season during moderate to heavy rainy season. Zamidi *et al.* (2012) reported prolonged spawning season of *E. tetradactylum* from March to September in Malacca coastal waters of Malaysia. Pember *et al.* (2005) reported peak spawning season of *E. tetradactylum* from October to December in north-western Australia. Observations on increasing trend in gonadosomatic index and the presence of mature males and females from May to November indicate that *E. tetradactylum* is a multiple spawner with asynchronous ovary having prolonged breeding season with a peak breeding season that coincides with monsoon season in Gujarat.

The study provides a comprehensive account of the reproductive biology of fourfinger threadfin *E. tetradactylum*, from the coast of Gujarat, north-eastern Arabian Sea, India. This is the first detailed report from Indian waters that provides a complete assessment, incorporating macroscopic and histological observations, filling gaps in knowledge regarding key reproductive parameters such as sex ratio, length at first maturity, length at first sex change, fecundity and reproductive seasonality of the species with an adequate sample size. The findings enhance scientific knowledge crucial for sustainable management, conservation, broodstock development and captive seed production for mariculture.

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