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## Reproductive Aspects of the Black Pomfret, *Parastromateus niger* in the Kuwaiti Waters of the Arabian Gulf

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### Abstract

The reproductive activities of the male and female black pomfret *Parastromateus niger* in Kuwaiti waters were investigated from October 2003 to September 2005. Analysis of seasonal variations in the gonadosomatic index (GSI) during the two-year study period revealed high values from February to September, suggesting that the black pomfret has a prolonged spawning season, from February to September. GSI fluctuations correlated positively with rising water temperatures in Kuwait from low values in both parameters in January to high values in February/March ( $r = 0.836$ ,  $p < 0.05$  for males and  $r = 0.764$ ,  $p < 0.05$  for females), suggesting that temperature plays a role in triggering spawning in both the sexes. Analysis of seasonal distribution of maturity stages for the two years revealed the presence of ripe/running males and females from February to September, thus confirming the spawning periodicity revealed through the analysis of fluctuations in the GSI. Macroscopic and microscopic studies of maturity stages revealed six stages in the males and seven in the females. The logistic function based on pooled data for the two years revealed that the minimum size at sexual maturity ( $L_{50}$ ) was attained at a size of 30.9 cm SL in males ( $r^2 = 0.284$ ) and 36.5 cm SL in females ( $r^2 = 0.355$ ). The ratio of males to females in monthly samples did not depart significantly from the hypothetical 1:1 during the entire study period ( $\chi^2 = 61.9$ , d.f. = 11,  $p < 0.05$ ). Total fecundity ranged from 71 305 in a fish measuring 39.8 cm SL and weighing 1 572.5 g, to 3 895 449 in a 49 cm SL and 2 630 g fish, with a mean of 1 216,734 eggs. Positive correlations were found between fecundity and ovary-free body weight, standard length and ovary weight, and a negative one with egg size. The average relative fecundity was 948 eggs/g ovary-free body weights, which was neither a function of fish standard length nor ovary-free body weight.

### Introduction

The black pomfret, *Parastromateus niger* (Carangidae), locally called *Halwayah*, is widely distributed in coastal waters of India (Sivaprakasam 1965; Rao 1973; Pati 1983; Kulkarni et al. 1991), in the Sea of Japan (Yukio et al. 1992) and along the eastern margin of the Indian Ocean through to the Arabian Gulf (Bishop 2003). It is found on the continental shelf, usually over muddy bottoms during the day, rising to the surface

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at night, often in large schools, swimming on their sides (Carpenter et al. 1997). It is of commercial interest not only in Kuwait but also throughout the Arabian Gulf. Annual catches of black pomfret from Kuwait have decreased from 290 tons in 1995 to 50 tons in 2003, and increased to 122 tons in 2004. The average annual catch is 150 tons. Black pomfret contributes about 3.1% to Kuwait's annual total fish catches (Ministry of Planning 2005).

Information on any aspect of the biology of this commercially important fish from the Arabian Gulf region is scarce (Dadzie 2007; Dadzie et al. 2007). Limited information is also available from Indian waters (Sivaprakasam 1965; Rao 1973; Pati 1983). In view of the continued importance of black pomfret to the commercial fishery in Kuwait, coupled with the scarcity of information on its biology both locally and regionally, the present study on aspects of its reproductive biology was undertaken, specifically to investigate the: (i) seasonal fluctuations in the gonadosomatic index (GSI), (ii) relationship between maturation pattern and temperature, (iii) maturity stages, (iv) seasonal distribution of maturity stages (v) size at maturity, (vi) sex ratio and (vii) fecundity.

### Materials and Methods

Fresh samples of black pomfret were collected from commercial gillnet catches in the northern part of the Kuwaiti waters of the Arabian Gulf (Fig. 1), during a 24-month sampling period, from October 2003 to September 2005.

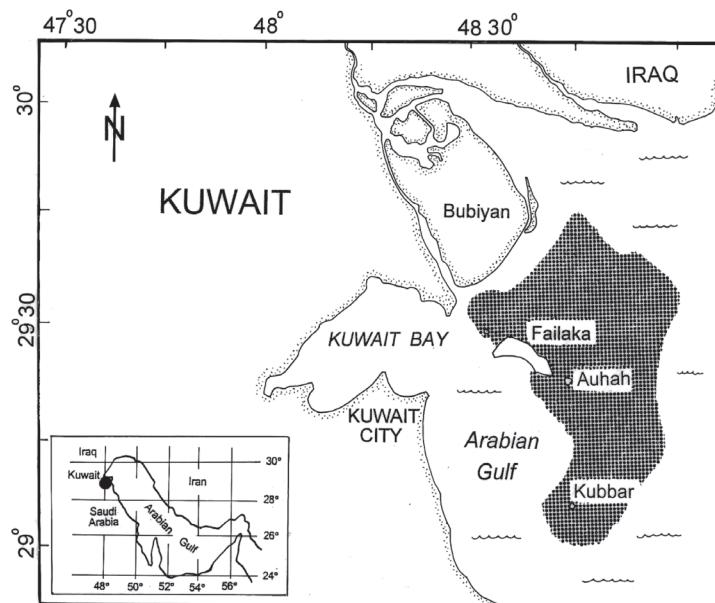


Figure 1. Map of Kuwait showing the study area (hatched)

The nets used were 1000-2500 m in length, with a stretched mesh size of 13.8 cm. They were set 2-5 km offshore at depths ranging from 7 to 15 m. They were set at dawn between 03:00 and 05:00 h, and raised for the collection of the fishes between 13:00 and 14:00 h. The vessels with black pomfret catches docked by 15:30 h, and study samples were obtained within 2 h and kept on ice.

The total length (cm), standard length (cm) and body weight (g) of each fish were recorded upon arrival in the laboratory. All the sampled fish were then dissected and their ovaries removed, weighed (to the nearest 0.001g) and fixed in Bouin's fixative for a minimum of 48 h. Large ovaries were cut into pieces before fixing to allow maximum penetration of the fixative. They were then dehydrated in alcohol, cleared in toluene and infiltrated with and embedded in paraffin wax. Sections of 5  $\mu\text{m}$  were stained in hematoxylin and counterstained with eosin for studies of the histological changes and maturity stages during the annual reproductive cycle and also to confirm the stages delineated by macroscopic characteristics.

The gonadosomatic index (GSI) was calculated using the formula:  $\text{GSI} = \text{Gonad weight} / \text{Ovary-free body weight} \times 100$ . The frequencies of the various maturity stages and the monthly variations in the GSI were used to study the maturation pattern and the extent of the breeding season. Mean monthly temperature values of the Kuwaiti waters covering the period of the study, obtained from the Kuwait Environmental Authority, were used to investigate the possible effects of temperature on the reproductive pattern of black pomfret in Kuwaiti waters. The size at maximum reproductive capacity, when 50% of the fish were mature ( $L_{50}$ ), was investigated separately for each sex and pooled data for the two years were fitted to a logistic curve using SPSS 12.0. The ratio of females to males in monthly samples was determined separately for each year and the results tested statistically ( $\chi^2$  test of homogeneity).

Mature ovaries, dissected from females during the spawning season (February to August), were used for fecundity studies. They were fixed for several weeks in Gilson's fluid, teased apart and vigorously shaken from time to time to separate the eggs. Total fecundity, defined as the standing crop of advanced yolked oocytes in the ovary (Hunter et al. 1992) was estimated for 107 fish by the gravimetric method (Kipling & Frost 1969), based on five 1-g sub-samples. Relative fecundity, defined as fecundity divided by female weight (Hunter et al. 1992), was also determined. The relationships of total fecundity to ovary-free body weight, fish standard length, and ovary weight and egg size were determined by regression analysis. Data on relative fecundity were regressed on fish standard length and ovary-free body weight to determine the relationships of these two parameters to relative fecundity.

## Results

### Seasonal fluctuations in the gonadosomatic index

The GSI profiles for males and females during the two study cycles (October 2003 - September 2004 and October 2004 – September 2005) were remarkably similar. Briefly, after a quiescent period from October to January, characterized by low GSI values, mean GSI values appeared to increase from February reaching a peak in June 2004 (Fig. 2a) and in March 2005 (Fig. 2b) for males.

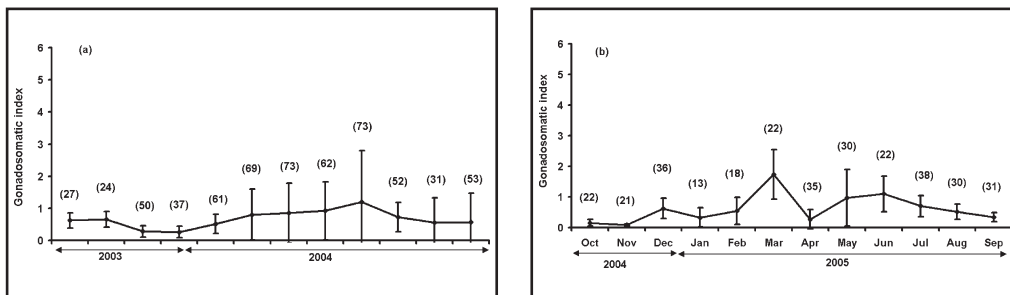


Figure 2. Seasonal fluctuations in gonadosomatic indices in male *P. niger*: (a) from October 2003 to September 2004, (b) from October 2004 to September 2005. Figures in parentheses indicate sample size

In the females, a minor peak was observed in March in both years and a major one in June and July 2005 (Fig. 3a and b), before declining from September.

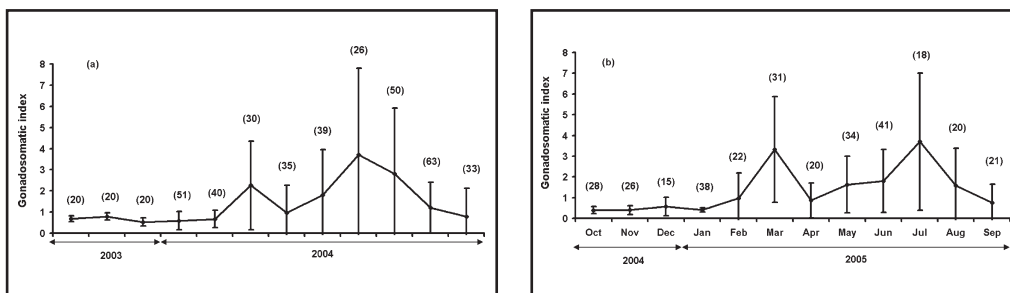


Figure 3. Seasonal fluctuations in gonadosomatic indices in female *P. niger*: (a) from October 2003 to September 2004, (b) from October 2004 to September 2005. Figures in parentheses indicate sample size.

### Relationship between maturation pattern and temperature

The maturation pattern, investigated through the analyses of the seasonal changes in GSI, was correlated with fluctuations in water temperature values during the study period. Two distinct phases in water temperature fluctuations were observed in Kuwaiti

waters during both the first and second cycles - a reduction from October 2003 to January/February, and an increase from January/February to September (Fig. 4a and b).

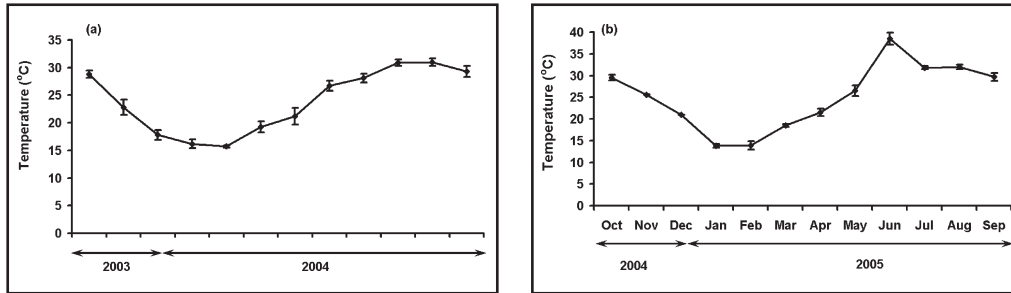


Figure 4. Monthly temperature values from Kuwaiti waters: (a) from October 2003 to September 2004, (b) from October 2004 to September 2005

Pooled data for the two years indicated very strong positive correlations between temperature values and GSI from the pre-spawning month (January) to the early months of spawning (February/March) ( $r=0.835$ ,  $p<0.05$  for males and  $r=0.764$ ,  $p<0.05$  for females).

**Maturity stages**

Based on morphological and histological characteristics, six maturity stages were identified in male (table 1) and seven in female (table 2) black pomfret.

**Seasonal distribution of maturity stages**

From October to January of both years, males and females in Stage I (immature), Stage II (developing) and Stage III (maturing) dominated the samples (Figs 5a and 6a).

This period coincided with the phase of reducing water temperatures depicted in Fig. 4. From February to September also of both years, the samples were dominated by

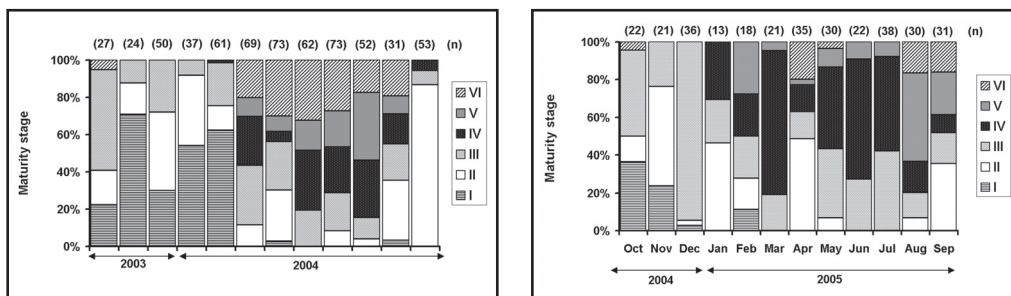


Figure 5. Seasonal distribution of maturity stages in male *P. niger*: (a) from October 2003 to September 2004, (b) from October 2004 to September 2005. Figures in parentheses indicate sample size.

Table 1. Macroscopic and histological characteristics of the maturity stages of male *P. niger*

Maturity stage	Macroscopic description	Histological description
I) Immature virgin/ Recovering spent	Testis in the form of two tiny transparent threads, occupying about 29% of the peritoneal cavity. Sex determination with unaided eye impossible.	Presence of primary germ cells (PGC), spermatogonia (SG) of subsequent generations, primary spermatocytes (PS) and secondary spermatocytes (SS).
II) Developing virgin /Recovering spent	Testis increases in size up to about 53% of the peritoneal cavity and takes on a light-brownish hue.	Presence of lobules filled with PGC and SG with increasing number of PS and SS. Few cysts containing spermatids (ST) also appear.
III) Maturing	Testis further increases in size, becomes dirty-white and occupies about 58% of the body cavity.	Increasing numbers of cell types, especially PS, SS, numerous ST as well as those recruiting into spermatozoa (SZ) confined within roundish or elongated lobules.
IV) Mature	Testis enlarged, turgid and whitish, occupying about 69% of the body cavity.	Lobules containing SZ increase in size as a result of formation of large numbers of SZ. However, interlobular spaces containing intermediate cell types still present.
V) Ripe/Running	Testis fully developed, highly vascularized, creamy-white in colour and occupies about 74% of the peritoneal cavity. Milt runs freely with a slight pressure on the peritoneum.	Both the roundish and elongated lobules are now packed with SZ. Very little evidence of interlobular spaces containing intermediate cell types.
VI) Spent	Both partially and fully shrunken testes present, occupying about 59% of body cavity.	Some SZ present in partially shrunken testis, but empty spaces characterize fully shrunken testis.

Table 2. Macroscopic and histological characteristics of the maturity stages of female *P. niger*

Maturity stage	Macroscopic description	Histological description
I) Immature virgin/ Recovering spent	Ovary small, thread-like and of equal length. It is translucent in immature virgins and reddish in recovering spends due to strong vascularization. It occupies about 39% of the body cavity.	Spaced ovigerous folds oriented towards the centre of the ovary containing oogonia (OG) and early perinucleolar stage oocytes (EPO) present.
II) Developing	Ovary increases in size, pale-yellow to dark pink in colour and occupies about 50% of the peritoneal cavity.	Ovary filled with EPO and late perinucleolar stage oocytes (LPO).
III) Developed/ Maturing	Ovary increases rapidly in size, yellowish, and occupy about 58% of the body cavity. Sex differentiation easily discernible.	Primary vitellogenic stage oocytes containing lipid vesicles in the cytoplasm appear in the ovary, indicating beginning of vitellogenesis.
IV) Maturing	Large ovaries containing yellow oocytes, occupying 69% of body cavity. Eggs are visible through the thin ovary wall.	Rapid increase in quantities of lipid vesicles. Appearance of secondary vitellogenic stage oocytes containing yolk granules. The latter and the lipid vesicles increase rapidly in size forming yolk globules and oil droplets.
V) Mature	Ovary swollen, maximally distended and yellowish, occupying 80% of body cavity. Eggs clearly visible throughout the thin ovary wall. A network of blood vessels surrounds the organ.	Presence of tertiary vitellogenic oocytes in which both yolk globules and oil droplets have increased considerably in size, and the latter coalescing.
VI) Spawning	Ovary very large, yellowish-red in colour, occupying about 94% of the body cavity, with an increased vascularization. Eggs freely extrude upon slight application of pressure on the peritoneum.	Numerous migratory-nuclear oocytes and hydrated oocytes are present in the ovary.
VII) Spent	Ovary reddish and flaccid, occupying about 66% of the body cavity. In totally spent females, it is shrunken, but in partially spent ones eggs meant for subsequent spawning(s) are present.	Ovary of totally spent females contain numerous post-ovulatory follicles at different stages of degeneration, atretic oocytes and a reserve stock of oogonia and perinucleolar stage oocytes; ovary of partially spent female contain, additionally, oocytes in different vitellogenic stages.

Stage IV (mature males and maturing females), Stage V (ripe/running males and mature females), and Stage VI (spent females) fish (Figs 5b and 6b). This period coincided with rising water temperature phase in Kuwait.

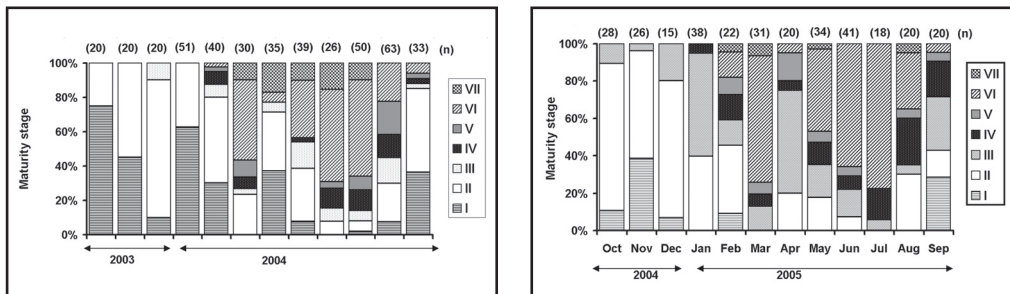


Figure 6. Seasonal distribution of maturity stages in female *P. niger*: (a) from October 2003 to September 2004, (b) from October 2004 to September 2005. Figures in parentheses indicate sample size

### Size at maturity

Maturing testis (Stage III and above) and maturing ovaries (Stage III and above) were considered mature for the determination of minimum size at sexual maturity. Pooled data covering the two-year study period indicated that males mature slightly earlier than females. Size at maturity ranged from 15 to 32 cm SL in males and 20 to 42 cm SL in females. In length groups larger than 17.5 cm SL over 50% of the males were mature (Fig. 7a); in length groups greater than 29 cm SL over 50% of the females were mature (Fig. 7b).

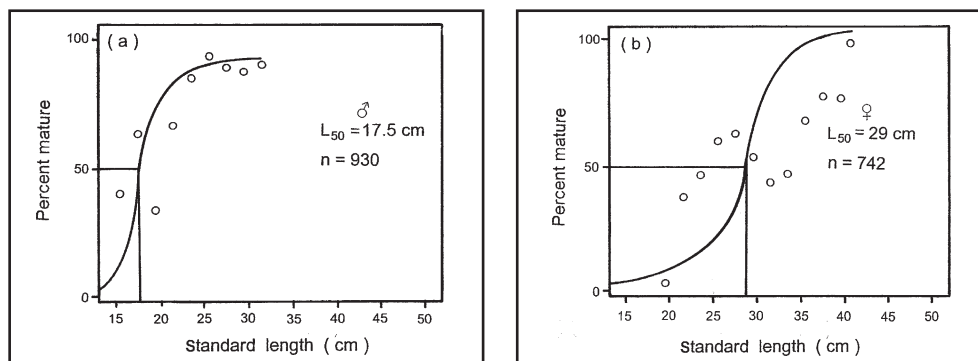


Figure 7. Logistic curve for determining size at sexual maturity in *P. niger*

The smallest mature males in the sampled population belonged to the 16.5-18.4 cm length class and only 5.6% of them were mature. The females started maturing at a length class of 18.5-20.4 cm, and only 3.1% of them were mature. The logistic function



revealed that the minimum size at sexual maturity ( $L_{50}$ ) was attained at a size of 30.9 cm SL in males ( $r^2 = 0.284$ ) and 36.5 cm SL in females ( $r^2 = 0.355$ ).

### **Sex ratio**

Out of 1 036 specimens sampled during the first year of the study, 612 were males and 424 were females, giving an overall sex ratio of 1.4:1 which did not deviate significantly from the hypothetical distribution of 1:1 ( $\chi^2 = 61.9$ ; d.f. = 11;  $p < 0.05$ ). During the second year, 627 specimens were sampled, out of which 315 were males and 312 were females, giving an overall sex ratio of 1.02:1, which also did not deviate significantly from the hypothetical 1:1 ( $\chi^2 = 46.9$ ; d.f. = 11;  $p < 0.05$ ).

### **Fecundity**

Total fecundity (TF) based on 107 mature females varied widely even among fishes of the same size. It ranged from 71 305 (for a 39.8 cm SL female weighing 1 572.5 g) to 3 895 449 (for a 49 cm SL female weighing 2 630 g), with a mean of 1 216 734 eggs. At 95% confidence limits, linear relationships were found between fecundity and: (a) ovary-free body weight ( $p < 0.05$ ;  $r^2 = 57.5\%$ ), (b) standard length ( $p < 0.05$ ;  $r^2 = 59.2\%$ ), and (c) ovary weight ( $p < 0.05$ ;  $r^2 = 70.8\%$ ). The  $r^2$  values revealed that the relationships were strong, the strongest being that between fecundity and ovary weight. In contrast, fecundity and egg size revealed a negative correlation ( $p > 0.05$ ;  $r^2 = 0.9\%$ ). Relative fecundity (RF) was 948 eggs/g ovary-free body weight, and did not reveal any significant relationship with either standard length ( $p = 0.023$ ;  $r^2 = 4.9\%$ ) or ovary-free body weight ( $p = 0.439$ ;  $r^2 = 0.6\%$ ).

## **Discussion**

Information on the maturation pattern and spawning of black pomfret in the Arabian Gulf does not exist. Within the region, however, reports are sparse and disparate and are all from Indian waters. Sivaprakasam (1965) observed mature fish from April till November, but ripe fish were recorded only in September, while actual spawners were found in September and October in Saurashtra waters. In view of the occurrence of spent fish in August, the author concluded that spawning had already started in July. These observations are different from the two-month spawning duration (February and March) reported from the Godavary Estuary (Rao 1973). In the Bay of Bengal, the species spawns from March to June (Pati 1983). Contrary to all the above reports, the present study has revealed that black pomfret has an extended spawning periodicity in the Arabian Gulf, beginning in February and ending in September. The evidence for this was derived from the presence of both males and females in the ripe/running condition (Stage V) in the samples from February till September. After a quiescent period from October till January, the increase in the GSI in both sexes from February,

till their decline in September, yields further evidence in support of this claim. In the only sympatric species studied from the Kuwaiti waters, *Pampus argenteus*, Dadzie et al. (1998, 2000) reported spawning from April to August although ripe males were encountered until September, while Almatar et al. (2004) observed spawning from mid-May to early October.

For the enhancement of gametogenesis leading to maturity and spawning, the role of temperature has been recognized (Ahsan 1966; de Vlaming 1974; Asahina & Hanyu 1983; Summers 1996). In the present study, the spawning season of black pomfret in Kuwait coincided with the period of increased sea temperatures. Furthermore, the strong positive correlation observed between the annual variations in GSI and sea temperature confirms that temperature, either alone or in combination with other unknown factors, triggers spawning in the species, similar to the observations found in *Acanthopagrus latus* in Kuwaiti waters (Abou-Seedo et al. 2003).

The determination of minimum size at sexual maturity based on pooled data for the two years revealed that minimum maturity in black pomfret from Kuwait is attained at a larger size in females than in males, similar to reports from Indian waters (Sivaprakasam 1965; Pati 1983). The ratio of males to females in monthly samples, which did not depart significantly from the hypothetical 1:1, confirms the observations also from the Indian representatives of the species (Sivaprakasam 1965; Pati 1983).

Fecundity in fishes characteristically varies considerably among individuals of the same size and age (Mathur & Ramsey 1974; Emery & Brown 1978; Dadzie et al. 2000, 2003), similar to the results observed in the present study. At the interspecific level, this may be due to intra-seasonal changes in the rhythm of egg laying as some fish may have shed many successions of eggs, some only a few, and others not at all (Mathur & Ramsey 1974; Emery & Brown 1978). This is especially characteristic of batch spawners (Yamamoto & Yamazaki 1961; Watson et al. 1992), the category to which black pomfret belongs.

### Acknowledgements

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