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Reproductive biology of *Puntius denisonii* (Day 1865) – an endemic ornamental cyprinid of the Western Ghats of India

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ABSTRACT

A study on the reproductive biology of *Puntius denisonii* showed that the length at first maturity is 60-70 mm in males and 70-80 mm in females. The absolute fecundity ranged from 293 to 967 ova in a female of 85 -122 mm total length and 7-16.35 g body weight. There is no clear cut sexual dimorphism in the species, but a mature female can be identified by the slightly bulged belly and its characteristic behaviour. Ova diameter studies indicated that the fish spawns once in a year during a short period of definite duration. The spawning season of *P. denisonii* in the River Iritty was found to be from December to February. This species can be successfully bred under captive conditions during December-January.

Keywords: Ornamental fish, *Puntius denisonii*, Reproductive biology, Western Ghats

Introduction

Puntius denisonii (Day, 1865), commonly called red line torpedo barb or Denison's barb is an endemic ornamental fish of the Western Ghats of India, belongs to the family Cyprinidae, Order Cypriniformes. It is locally known as *chorakanni*, *chenkananjon* or *chorakaniyan* in Kerala. It is a much sought after fish in the international ornamental fish market and contributes to about 60% of the total export of ornamental fish from India. Presently it is being collected from the wild and exported. Due to the indiscriminate exploitation from the wild, the population of the species is dwindling. Though much is talked and discussed about its conservation, very little has so far been published about the reproductive biology of the species. Radhakrishnan and Kurup (2008) studied *Puntius denisonii* from the river Bharathapuzha and Solomon *et al.* (2011) studied the same species from rivers Chandragiri, Valapattanam and Chaliyar.

This paper presents the reproductive characteristics of *P denisonii* from the river Iritty, a branch of river Valapattanam in northern Kerala. The aim of the study was to contribute information on the spawning season, spawning frequency, sexual dimorphism, absolute as well as relative fecundity and length at first maturity of specimens collected from river Iritty.

The species grows to a maximum size of 24 cm (personal observation). Preliminary survey conducted by the authors on the distribution and stock assessment of this species has revealed that it is distributed in eleven rivers of Kerala *viz*., Chandragiri, Karingode, Kuppam, Valapattanam, Anjarkandipura, Kuttiyadi, Chaliyar, Chalakudy, Bharathapuzha, Bhavani and Pampa (Mercy *et al.*, 2010).

Materials and methods

Study area

Two hundred specimens of the species were collected for the present study from the river Iritty during October 2008 to September 2009. The specimens were brought to the laboratory, and the total length and weight of the fishes were measured nearest to 1.0 mm and 0.1 g respectively.

Sex was determined by external examination of the morphology of the gonads and by cursory examination under the microscope. After measurement, ovaries were carefully excised from the body cavity and preserved in Gilson's fluid (Simpson, 1951). Maturity stages of gonads were assessed (Qasim, 1973). A modified five stage key as given below was used for the females:

I: Immature virgins. Ovaries very thin, transparent occupying 25% of body cavity, ova very small and transparent.

II: Maturing virgins and recovered spent. Ovaries slightly enlarged, pale yellow, occupying at least 50% of body cavity. Ova rounded and translucent; yolk has begun to accumulate.

III: Ripening. Ovaries enlarged, hued pinkish yellow, occupying 75% of body cavity, ova opaque with distinct yolk drops.

IV: Ripe. Ovaries much enlarged, orange yellow, almost filling body cavity, ovary wall thin and fragile, expulsion of ova at slight pressure on the abdomen.

V: Spent. Ovaries shrunken and flaccid, flesh-coloured, occupying about 35% of body cavity, some residual ripe ova.

Length at first maturity

Length at first maturity (the length when 50% of the population is mature) was computed based on the maturation curve plot (Kagwade, 1968) with a total of 200 fishes ranging in size from 40-120 mm (for 45 females) and 40-150 mm (for 155 males).

Ova diameter studies, spawning season, fecundity and fecundity indices

During the initial quantification, ovaries belonging to all the stages of maturity were preserved in Gilson's fluid for ova diameter measurements. Sub-samples were taken from different parts of the ovary to eliminate any error due to differential distribution of ova stocks. To assess the spawning season of the species, three criteria were employed: occurrence of mature gonad stage in females, appearance of the eggs, embryos and larvae in the natural habitat and appearance of the smallest size groups of live fishes in the catch (De Silva *et al.,* 1985). Fecundity is the total number of ripe ova of the ovaries prior to spawning (Bagenal, 1978). Total number of ripe ova of nineteen ripe ovaries were counted to calculate the fecundity. Fecundity in relation to total length (cm), body weight (g), ovary length (cm) and ovary weight (g) of fish were estimated.

Results and discussion

Gonads

The mature testes occur as a pair of milky white elongated mass lying on either side of the intestine and lodged into the groove between the bladder and abdomen (Fig. 1a and b). The mature ovary is yellowish-orange with large-sized eggs. The fully mature ovary occupies nearly the entire body cavity. Both lobes of the mature ovary are equal in length and size (Fig. 2a and b)

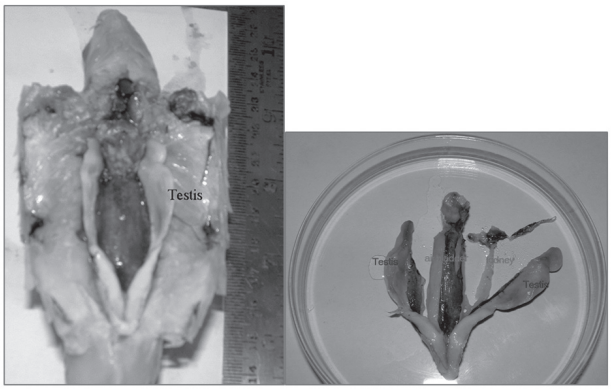

Fig. 1 a and b. Mature testes of *Puntius denisonii*

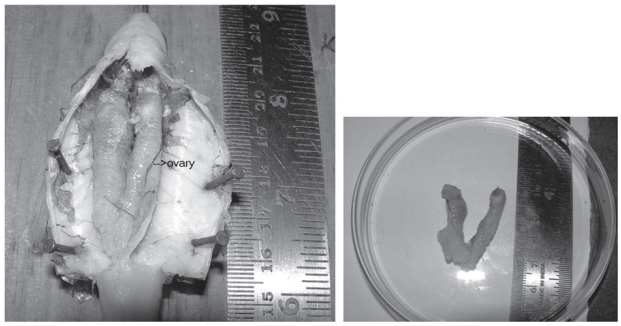

Fig. 2 a and b. Mature ovary of *Puntius denisonii*

Length at first maturity

Fish with gonads at stage III or above were considered mature. The length at first maturity was found to be 60-70 mm in males (Fig. 3) and 70-80 mm in females (Fig. 4). All male fishes of total length 90 mm and above and all females of total length 100 mm and above were mature. Males attained sexual maturity at a smaller size than the females. Similar observations have been made in other *Puntius* species like *P. melanampyx* (Harikumar *et.al.,*1994), *P. melanostigma, P. filamentosus* (Mercy *et al.,* 2005b,) *P.* (Seena *et al.,* 2012) and in other fishes from the Western Ghats like, *Danio malabaricus, (*Mercy *et al*., 2005a), *Nemacheilus triangularis, Nemacheilus semiarmatus* (Mercy and Jacob, 2004), *Pristolepis*

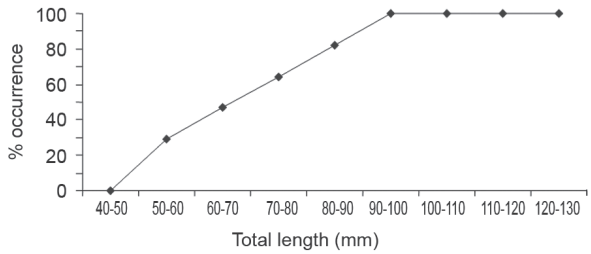

Fig. 3. Size at first maturity of male *Puntius denisonii*

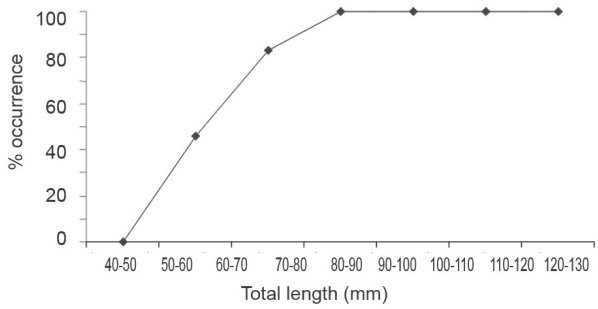

Fig. 4. Size at first maturity of female *Puntius denisonii*

marginata. (Mercy *et al*., 2002), *Chela fasciata* and *Garra mullya* (Mercy *et al.,* 2007). This variation in the length at first maturity may be related to ecological factors and food supply as reported by Keshava and Joseph (1988). Similar observations were also reported by Solomon *et al.* (2011) in *P. denisonii* collected from the rivers Chandragiri, Valapattanam and Chaliyar.

Fecundity and fecundity indices

The fecundity of 19 females in the length range of 85- 122 mm and body weight range of 7-16.35 g was determined. The absolute fecundity ranged from 293 to 967 ova. The relationship between absolute fecundity and total length, total body weight, ovary length and ovary weight are given in Table 1. Since the correlation coefficients estimated were found to be significant at 5% level, the relationships were assumed to be linear.

Table. 1. Relationship between fecundity and other variables in *Puntius denisonii.*

Parameter	Regression equation	Correlation coefficient
Total length (TL)	y = 91.428 x - 416.95	0.506383*
Weight of fish (WF)	y = 37.502 x + 111.85	0.584294*
Length of ovary (OL)	y = 247.97 x - 44.944	0.713008*
Weight of ovary (OW)	y = 654.39 x + 155.95	0.863535*

*Significant at 5% level

Solomon *et al.* (2011) reported that the absolute fecundity of *P. denisonii* is extremely low when compared to other cyprinids such as *P. sarana* (Chandrasoma and De Silva, 1981) and *Rasbora daniconius* (Nagendra *et al.,* 1981). *Puntius nigrofasciatus, P. cumingi* and *P. pleurotaenia* are also known to have a low absolute fecundity ranging between 151 and 638 for fish of length 46-64 mm (De Silva and Kortmulder 1976; Chandrasoma *et al.,* 1994, Harikumar *et al.,* 1994).

The relative fecundity was estimated to be 50.45 eggs per cm body length; 47.88 eggs per g body weight, 226.04 eggs per cm ovary length and 1049 eggs per g ovary weight. De Silva *et al*. (1985) also reported similar findings in six species of *Barbus,* indigenous to Sri Lanka. According to Radhakrishnan and Kurup (2008), the absolute fecundity of *P. denisonii* from river Kanjirampuzha, a tributary of river Bharatapuzha, is between 74 and 284 and relative fecundity is 9-24 per cm body length. However, the present study has shown that the absolute fecundity ranged from 293 to 967 and the average relative fecundity is estimated as 50.45 eggs per cm body length.

According to Solomon *et al*. (2011), the fecundity of *P. denisonii* is 27^{th} power of length and 10^{th} power of weight of the fish and hence the scaling exponent of the relationship of absolute fecundity with both total length and total weight in *P. denisonii* were significantly different from the values suggested by Euclidian geometry, and thus fecundity grows non-isometrically. However, the present study indicated that the fecundity of *P. denisonii* is cube power of length and weight of the fish, which indicates that the scaling exponent of the relationship of absolute fecundity with both total length and total weight of *P. denisonii* is not significantly different from values suggested by Euclidian geometry.

Sexual dimorphism

There is no clear cut sexual dimorphism in *P. denisonii*. But a person who is well acquainted with live fish can identify a mature female by observing its belly shape, which is slightly bulged, and from the behaviour of the female, which is extremely sensitive to even the smallest stress and dies immediately on capture from the wild or even when it matures in captivity (personal observation). In fact, it is quite difficult to get live mature female fishes for any experimental studies. This is one of the main reasons why many scientists and farmers working on this species for several years failed to develop its captive breeding technology.

Sex ratio

There has always been a male dominance, many times leading to an overall dominance of males in the sample, especially towards the breeding period of the fish. The proportion of females in the population appeared to be the lowest during the beginning of a breeding period. This trend might be indicative of migratory patterns pertaining to spawning activities in the populations. Chandrasoma and De Silva (1981) related changes in the sex ratio in *Barbus sarana* populations in a lacustrine environment to spawning migrations. It may be that females of *P . denisonii* also migrate to safe spawning grounds around the time when they become receptive. The non-availability of mature females in the population was the main hurdle encountered during the past years in attempts to breed the fish in captivity. This has also been reported by many stakeholders who have been trying to develop captive breeding technology for this species. The collectors have suggested (personal communication to the first author) that the female fishes remain in hiding in some inaccessible places during the period between November and February. Moreover, the females are extremely sensitive and any disturbance in the

habitat or the handling of the fish immediately leads to its mortality. The parental investment of females prior to spawning periods may be larger than that of males, thus rendering them more vulnerable during spawning as mentioned by De Silva *et al*. (1985). The possible role of other factors such as differential mortality of the sexes cannot be analysed from the present data. Radhakrishnan and Kurup (2008) reported that the sex ratio is 1:0.692. Solomon *et al*. (2011) opined that the skewed ratio in *P. denisonii* could be due to the differential habitat occupation of the sexes.

Ova diameter frequency studies

The ova diameter frequency distribution of ripe ovaries indicated that there are two distinct groups of eggs. The first mode is represented by the transparent immature stock and the second mode comprised of the largest ripe eggs which are to be spawned immediately (Fig. 5).

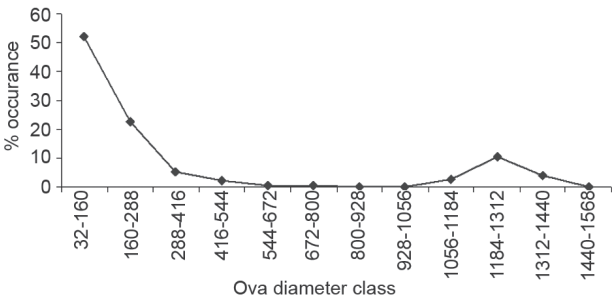

Fig. 5. Percentage frequency of ova diameter in a ripe ovary of *Puntius denisonii*

The ova diameter ranged from 32 to 288 µm in the immature stocks. The ripe stock had ova diameter range of 928 – 1440 µm. There was a large stock of immature ova constituting an average of about 83% of the total ova count which ranged in diameter from 32 to 288 µm. The ripe stock constituted an average of about 17%, with ova size going up from 928 µm and with a mode at 1184 - 1312 µm diameter class. The maximum diameter of ova recorded during the present study is 1440 µm. An intermediate stage of ripening mode was not apparent in the present study. There was always the presence of large percentage of immature stock and a distinguishable stock of ripe ova in the ripe ovary with clear-cut modes, without an apparent intermediate stock. This ova-stock distribution picture is indicative of spawning taking place once in a year during a short period of definite duration (Karekar and Bal, 1960). The relatively high proportion of reserve oocytes in the ovaries of barbs has also been noted earlier (Al-Daham and Bhatti, 1979).

Spawning season

Several independent observations by the first author on the breeding season of *P. denisonii* during the last five years indicate that breeding takes place during December-March. Fishes collected during March-April represented only ovaries in spent condition. Fully mature females could be obtained during November-December. During January-February, natural spawning was personally observed by the authors in the natural habitats of river Iritty and eggs, embryos as well as larvae were seen in the spawning ground. The smallest size group of the fish larvae appeared in the collection during the months of March-April. Hence the spawning season of *P. denisonii* appears to be during the period of December to February, as evident from the presence of mature females observed in the river, the appearance of the egg, embryo and larvae in its natural habitat, and the appearance of smallest size groups in the catch. The same has also been observed in the rivers Valapattanam, Chaliayar and Karingode. Similar observations have been made by De Silva *et al.* (1985) in six barb species indigenous to Sri Lanka. Radhakrishnan and Kurup (2008) however, have reported that *P. denisonii* of River Bharathapuzha breeds during the months of July and October.

The climate of North Kerala is subequatorial and the south-west monsoon brings heavy rains from June to August and the north-east monsoon brings another rainy spell from October to November. The water current velocities are higher and periodically very high during rainy season. *P. denisonii* apparently seeks quiet water for spawning as barbs generally do (Schut *et al.,* 1984; Harikumar *et al.* (1994). Hence it may be assumed that the adaptive significance of dry season spawning of this species is to avoid strong currents as reported by Harikumar *et al*. (1994) in the case of *Puntius melanampyx.*

During the months from December to March, the water level in the rivers of Kerala is very low and water logging occurs in certain pockets where it is comparatively deeper and temperature is lower (22-24 ^{0}C). It appears quite likely from field observations that *P. denisonii* undertakes small spawning migrations to these areas. It has been noted in the course of observations in the present study that *P. melanampyx, P. filamentosus* and *P. denisonii* co-exist and breed in the same habitat in the same season, and the larvae of all the three species look alike with dark bands across the body. It is quite difficult to distinguish between the larvae. In the case of *P. filamentosus* and *P. denisonii,* the cross-bands are retained for about one month. They gradually fade to a single spot at the caudal peduncle in the case of *P. filamentosus* whereas they are completely absent in *P. denisonii*. De Silva *et al.* (1985) have also reported that among *Puntius* species in Sri Lanka, seasonal and perennial breeders share the same macrohabitat. Successful breeding of this species under captive conditions during December-January has already

been reported earlier by the authors (Mercy *et al.,* 2010). The information presented in this paper throws light on reproductive biology and spawning season as well as requirements of *P. denisonii,* and is therefore will be useful for undertaking captive breeding programmes and towards the conservation as well as management of natural population of the species.

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