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Western Ghats Special Series

Reproductive biology of *Puntius denisonii*, an endemic and threatened aquarium fish of the Western Ghats and its implications for conservation

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Abstract: This study presents fundamental information on the reproductive biology of *Puntius denisonii*, an endemic and threatened aquarium fish of the Western Ghats Hotspot. Results are based on the observations from three river systems, Chandragiri, Valapattannam and Chaliyar. Maximum observed total length in *P. denisonii* was 162mm and 132mm for males and females, respectively. Males attained sexual maturity at a lower size than females with mean size at first maturity determined as 85.33±1.52 mm for males and 95.66±1.15 mm for females. *Puntius denisonii* spawned from October to March with minor differences in the peak breeding months between the three river systems, which were studied. Sex ratio deviated significantly from 1:1 and was skewed in favour of males. Absolute fecundity varied from 376 (fish of 102mm total length) to 1098 (fish of 106mm total length) eggs. Currently, the closed seasons for *P. denisonii* have been put in place during June, July and October based on the (mis)assumption that the species breeds during these three months. However, the results of the present study have helped us to understand more about the reproductive biology of the species so as to recommend more appropriate seasonal closures. The months from October until March need to be designated as a closed season for protecting the breeding population of *P. denisonii*.

Keywords: Conservation, endemic fish, *Puntius denisonii*, reproduction, threatened, Western Ghats.

INTRODUCTION

Unsustainable collection of endemic freshwater fish for the aquarium trade is an emerging conservation issue in the tropics, which has resulted in the population decline of several species such as the Asian Arowana *Scleropages formosus* (Rowley et al. 2009), Silver Arowana *Osteoglossum bicirrhosum* (Moreau & Coomes 2006), Celestial Pearl Danio *Danio margaritatus* (Roberts 2007) and Bala Shark *Balantiocheilos*

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melanopterus (Ng & Tan 1997). Nevertheless, wild caught aquarium fish industry receives little attention from ichthyologists, local governments and conservation organizations throughout the world, with very little research, and no legislative controls (Moreau & Coomes 2007; Rowley et al. 2009).

The Western Ghats (WG), an exceptional Hotspot of freshwater fish diversity and endemism in peninsular India (Kottelat & Whitten 1996; Dahanukar et al. 2004) is an important region for aquarium fish collections (Tlusty et al. 2008). More than a hundred species including several threatened endemics are currently collected and exported from this region (Raghavan 2010). Similar to other parts of the world, aquarium fish collections in WG are open access and unregulated, raising concerns about their eco-biological impact (Raghavan 2010). Several endemic species are known to be facing serious population decline due to indiscriminate collections for the trade (Kurup et al. 2004; Raghavan et al. 2009).

One such endemic species, which is currently considered to be under severe threat from the aquarium pet trade is the Denison Barb (AKA Red Lined Torpedo Barb and Miss Kerala), *Puntius denisonii*, a small- to medium- sized cyprinid having an extremely restricted distribution in the southern WG (Prasad et al. 2008). Due to its limited distributional range in the southern WG and declining populations, *P. denisonii* was assigned Vulnerable species status in the IUCN Red List (Devi & Boguskaya 2009). The recently completed IUCN Freshwater Biodiversity Assessments in the WG has categorised this species as Endangered (Ali et al. 2010). Nevertheless, this species is poorly known with no information on its micro level distribution, life history, ecology and demography (Raghavan et al. 2010). The objective of this study was to understand the reproductive biology of *P. denisonii*, and discuss its implications on the conservation of wild populations.

MATERIALS AND METHODS

Samples for the present study were purchased from aquarium fish collectors operating in three major rivers of the southern WG, viz., Chandragiri, Valapattannam and Chaliyar (Fig. 1) between December 2008 and November 2009. Fish were received live in packed polythene bags and euthanized immediately by immersing in ice-slurry. Subsequently they were preserved in 4% formaldehyde and transferred to the laboratory, where each individual was tagged, measured (Total Length T_L), weighed (Total Weight T_W) and sexed (by internal sexual characteristics or by examining gonads under a dissecting microscope). Gonads were subsequently removed, weighed (G_W) and preserved in 4% formaldehyde, while matured ovaries with visible eggs were preserved in Gilson fluid (100ml 60% alcohol, 800ml water, 15ml 80% nitric acid, 18ml glacial acetic acid, 20g mercuric chloride) to break down ovarian tissues.

Gonado somatic index (GSI) was calculated as 100 X G_W $(T_W\text{-}G_W)^{-1}$ and used to delineate the spawning season. The length at which 50% of male and female fish were in maturing stages III and IV was taken as the minimum length at first maturity (Bagenal 1978). Deviation from the expected 1:1 sex ratio was analyzed using chi-square test (Corder & Foreman 2009). Absolute fecundity (A_F) was estimated by weighing all the eggs in the ovary and also by counting three sub samples of eggs from different parts of the ovary. Relative fecundity (R_F) was calculated as T_F/T_W. Relationship of A_F with both T_L and T_W were determined by plotting the points on a log-log scale as these are expected to be allometric relationships described by a general power function $y = ax^b$, where y is the dependent variable, x is independent variable, b is the scaling exponent and a is the normalization constant (Kharat et al. 2008). A least square line was fitted to the scatter of the data and the significance of the relationship was determined from coefficient of determination (R^2) and uncertainty in the prediction of the exponent by calculating its standard error.

RESULTS

Of 1,080 fish analysed, 792 (73.33%) were mature, composed of 570 males (52.77%) and 222 females (20.55%). Sex ratio of *P. denisonii* from all three rivers deviated significantly from the expected 1:1 and was extremely skewed in favour of males (Table 1). GSI in all three river systems peaked during October to March with minor differences between rivers (Fig. 2). Peak maturity of *P. denisonii* in Chandragiri and Valapattannam rivers were observed during December

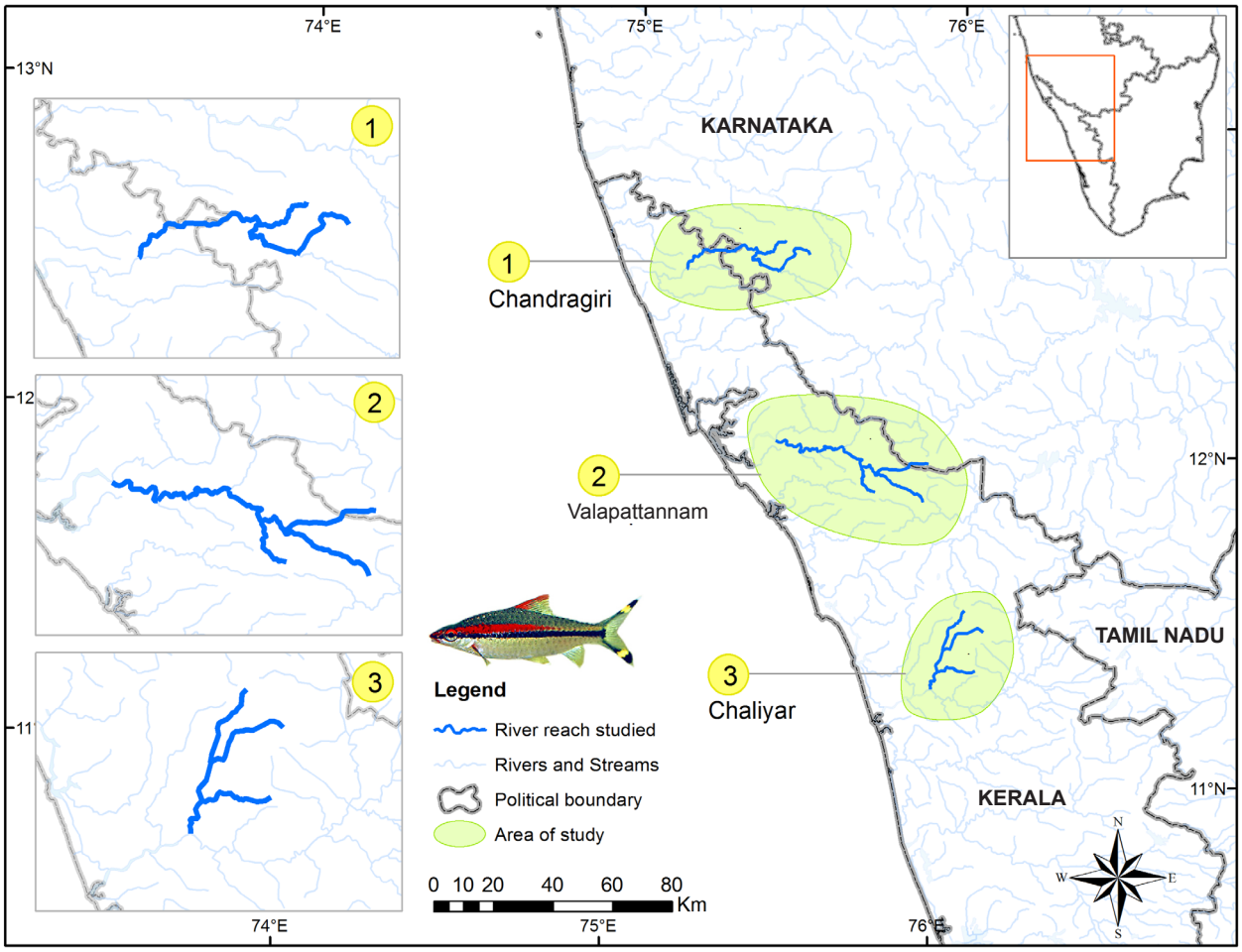

Figure 1. The three river systems from where *P. denisonii* were collected in southern India

and, in the Chaliyar River during February. No temporal variation in spawning season could be observed even though the three rivers from where the fish samples originated were located at different latitudes (Fig. 1).

In *P. denisonii*, males start to mature earlier than females (Table 1). Mean sizes at first maturity was 85.33±1.52 mm T_L (male) and 95.66±1.15 mm T_L (females). Absolute fecundity (A_F) in *P. denisonii* from the Chandragiri River system varied from 376 (102mm T_L) to 1098 (106mm T_L) with a mean of 762.66±264.270 eggs/fish (n=12), while relative fecundity (R_F) was between 36.11 and 94.65 with a mean of 70.44±22.79 eggs. Although we obtained several fecund female specimens of *P. denisonii* from the other two rivers as well, they were released back into the stream without sacrificing for our study. This was done taking into consideration the threatened status of the species, and based on our assumption that the same

Table 1. Maximum observed length, minimum size at first maturity and sex ratio of *Puntius denisonii* from three river systems of Western Ghats

River	Max length (mm T_L)		Min size at maturity (mm T_L)		Sex ratio (M:F)	
	Male	Female	Male	Female	Ratio	Chi Square (χ^2)
Chaliyar	110	100	84	97	1:0.35	63.947*
Chandragiri	162	132	87	95	1:0.36	49.717*
Valapattannam	136	105	85	95	1:0.57	19.810*

* - P < 0.0001

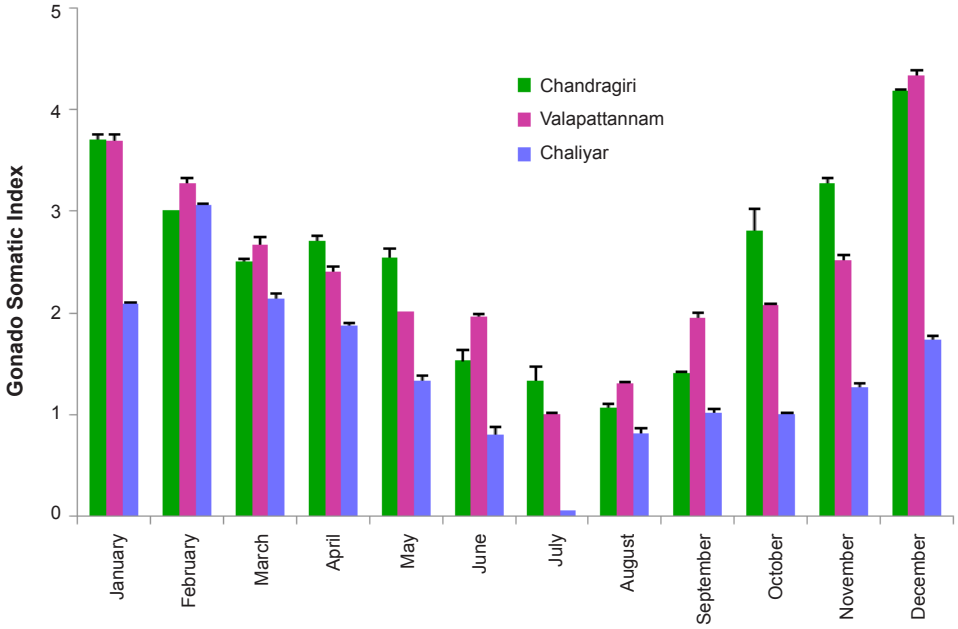

Figure 2. Annual changes in the mean Gonado Somatic Index (GSI) of *Puntius denisonii* from three river systems of Western Ghats (error bars denote standard deviation).

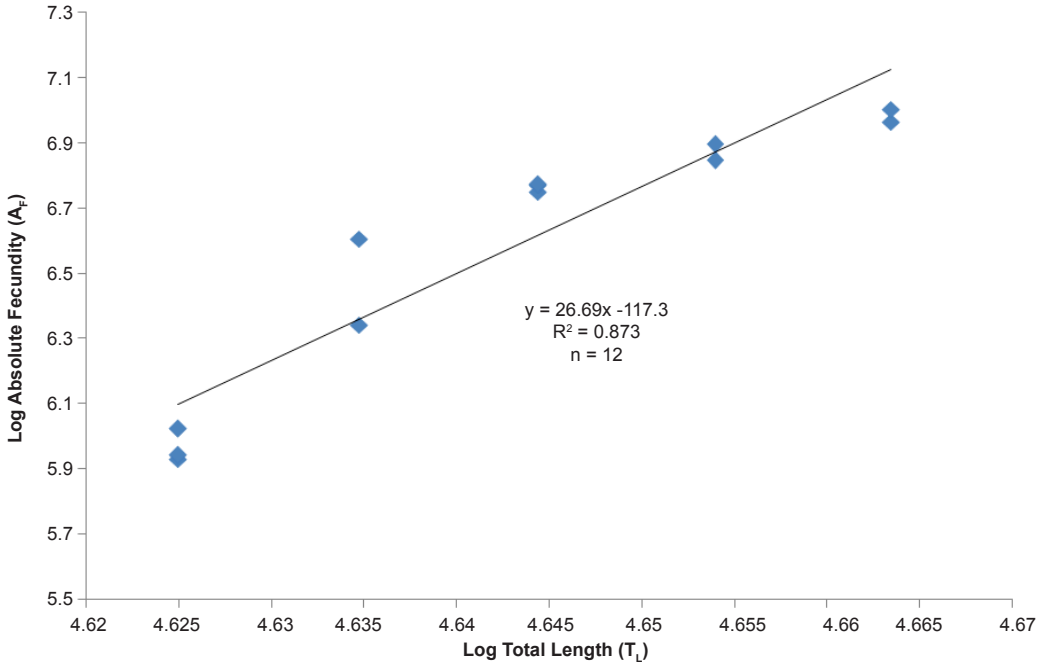

Figure 3. Relationship between Total Length and Absolute Fecundity in *Puntius denisonii* from Chandragiri River

species may show similar range of fecundity between river systems. The relationship of absolute fecundity with total length was best explained as $\log A_F = 26.69 \log T_L - 117.3$ (Fig. 3) and the relationship of absolute fecundity with total weight was better explained as $\log A_F = 9.55 \log T_W - 16.10$ (Fig. 4).

DISCUSSION

Although sex ratio of a fish may deviate from the normal 1:1 due to a number of factors (Nikolsky 1963; Alp et al. 2003) extremely skewed ratios such as those observed in the present study are very rarely

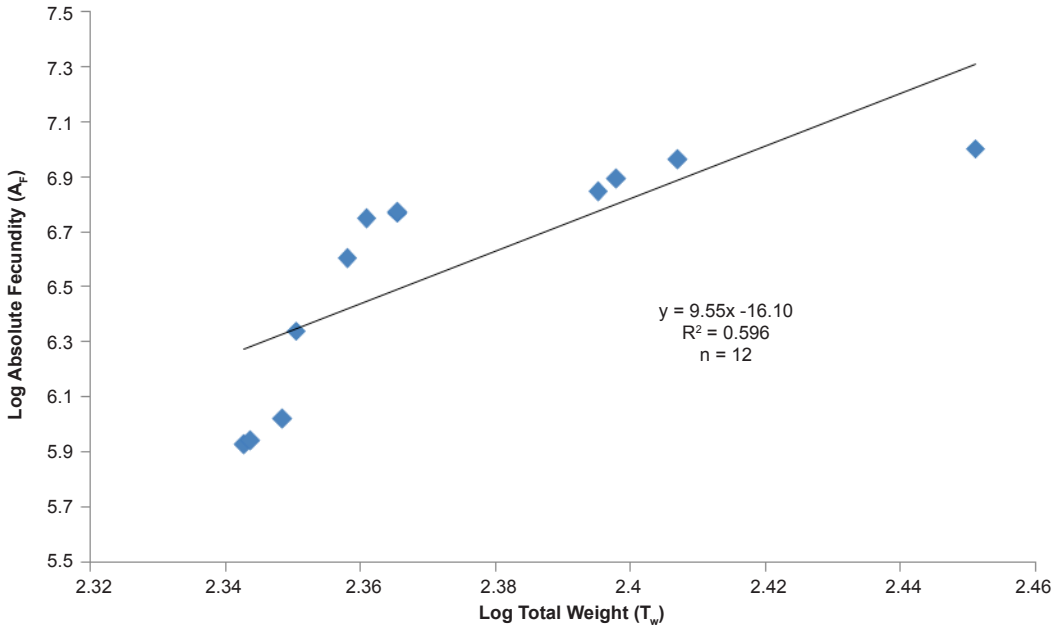

Figure 4. Relationship between Total Weight and Absolute Fecundity in *Puntius denisonii* from Chandragiri River

encountered. One possible reason for this skew in *P. denisonii* could be the differential habitat occupation of the sexes. i.e., females preferring deeper waters and therefore being less vulnerable to capture and males on the other hand living in shallow areas from where they are easily caught. Such differential habitat occupancy by sexes has been earlier observed in tropical fish (Macuiane et al. 2009; Lewis et al. 2005). Skewed ratios may also occur as a result of the differences in instantaneous natural mortality between sexes (Vincentini & Araujo 2003). However, there is no information on the demography of *P. denisonii* to support such an argument.

Results obtained in this study on the spawning season are contrary to the information in gray literature. *P. denisonii* was reported to spawn during June-August with mature specimens observed from May (Radhakrishnan & Kurup 2005). However, the annual dynamics of GSI from three river systems of WG observed in this study indicated that *P. denisonii* breeds during October to March.

As the first step towards conservation, the State Department of Fisheries in Kerala (India) has issued an order, restricting collection and exports of *P. denisonii* from the rivers of the region (Clarke et al. 2009). Several management measures including quotas, restrictions on gears, catch size, and a seasonal closure of fishery have been enforced (Mittal et al. 2009). Currently, the closed seasons for *P. denisonii* have been put in place during June, July and October (Clarke et al. 2009) based on the assumption that the species breeds during these three months. However, results of the present study provide hard evidence that this seasonal closure is mistimed and has been designed without proper understanding of the biology of this species.

Absolute fecundity of *P. denisonii* is extremely low when compared to other cyprinids such as *P. sarana* (Chandrasoma & de Silva 1981) and *Rasbora daniconius* (Nagendran et al. 1981). However, three endemic cyprinids threatened by aquarium collections in Sri Lanka, *P. nigrofasciatus*, *P. cumingi* and *P. pleurotaenia* are known to have a low absolute fecundity (151–638 for 46–64 mm T_L) (de Silva & Kortmulder 1976; Chandrasoma et al. 1994) similar to *P. denisonii*.

As the scale of the body increases the relationship depicting change in lengths and weights of different body parts change as allometric relationships. As per Euclidian geometry, the lengths of two tissues should show an exponent of one and the relationship depicting change in length versus weight should show an exponent of 1/3 depicting isometric relationships. Kharat et al. (2008, p. 13) suggested that if the volume of each egg is constant, then the fecundity should scale as unity with the ovary volume and as a result,

at a constant density, the fecundity should change as a cube of length of the fish and as unity with weight of the fish under isometry. On the contrary, in our analysis the fecundity changed as 27th power of length and 10th power of the weight of the fish. Our results show that 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 the fecundity grows non-isometrically. As a result, the larger fish (length and weight) have drastically more fecundity then the slightly smaller individuals. Thus, larger specimens contribute more to reproduction in the species and the removal of larger individuals from a population will have a drastic impact on the demographics and subsequently on the status.

The peculiar characters of reproduction including an extremely low absolute fecundity and a skewed sex ratio will undoubtedly hamper natural recruitment, influence population dynamics and lead to low population levels in *P. denisonii*. This cyprinid may therefore be unsuited for large scale collections for the pet trade. The present study has also revealed that closed seasons, the most important conservation plan for *P. denisonii* implemented by the local government in Kerala is wrongly timed, and has little or no impact on the protection of wild stocks. There is hence an urgent need for re-designing conservation strategies for the species based on biological information such as those generated in this study. The closed season for protecting the breeding population of *P. denisonii* in the rivers of northern Kerala should be put in place from October to March.

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Author Contributions: BP, AA and RR designed the study; SS, MRR and FB carried out the field and laboratory work; AA, JT and RR carried out the analysis; RR wrote the manuscript; RR was the Principal Investigator of the projects from which the current manuscript originated.

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Malayalam Abstract:

പശ്ചിമഘട്ടത്തിലെ വംശനാശഭീഷണി നേരിട്ടുകൊണ്ടിരിക്കുന്ന ചോരക്കണ്ണിയാനെന്ന അലങ്കാരമത്സ്യത്തിന്റെ പ്രത്യുല്പാദന ജീവശാസ്ത്രത്തെക്കുറിച്ച് നടത്തിയ പഠനാന്തരം ഉരുത്തിരിഞ്ഞ അടിസ്ഥാന വിവരങ്ങളാണ് ഇവിടെ അവതരിപ്പിക്കുന്നത്. കേരളത്തിലെ ചന്ദ്രഗിരി, വളപട്ടണം, ചാലിയാർ എന്നീ ഉത്തരനദികളിലെ പ്രസ്തുത മത്സ്യങ്ങളിൽ നടത്തിയ നിരീക്ഷണങ്ങളിൽ ആൺ മൽസ്യങ്ങൾ പെൺ മത്സ്യങ്ങളെക്കാൾ വളരെ ചെറുവലുപ്പത്തിൽത്തന്നെ ലൈംഗികപക്വത കൈവരിക്കുമെന്നും പ്രഥമ പ്രജനനത്തിന് വേണ്ട ശരാശരി വലുപ്പം ആൺ-പെൺ മത്സ്യങ്ങൾക്ക് യഥാക്രമം 85.33 ± 1.52 mm, 95.66 ± 1.5 mm ആണെന്നും മനസിലാക്കാൻ സാധിച്ചു. പഠനവിധേയമാക്കിയ മൂന്ന് നദികളിലും ഒക്ടോബർ മുതൽ മാർച്ച് വരെയുള്ള കാലയളവിലാണ് ഈ മൽസ്യം പ്രത്യുല്പാദനം നടത്തുന്നതെന്നും മാതൃകാപരമായ ലിംഗാനുപാതത്തിൽ (1:1) നിന്നും വ്യതിചലിച്ച് ആൺ മൽസ്യങ്ങൾ കൂടുതലായി കാണപ്പെടുന്ന സ്ഥിതിവിശേഷം പ്രദർശിപ്പിക്കുന്നതായും കാണാൻ കഴിഞ്ഞു. ജൂൺ മുതൽ ഒക്ടോബർ വരേയുള്ള മൂന്ന് മാസക്കാലയളവിലാണ് പ്രത്യുല്പാദനം നടത്തുന്നതെന്ന അബദ്ധധാരണയിൽ പ്രസ്തുത കാലയളവിൽ കേരളത്തിലെ നദികളിൽ ചോരക്കണ്ണിയാനു 'കാലിക മത്സ്യബന്ധനനിരോധനം' നടപ്പിലാക്കിവരികയാണ്. ഈ സാഹചര്യത്തിൽ ചോരക്കണ്ണിയാൻറെ പ്രത്യുത്പാദന ജീവശാസ്ത്രവിവരങ്ങളിലേക്ക് വെളിച്ചം വീശുന്ന ഈ പഠനഫലത്തെ ആധാരമാക്കി പ്രസ്തുത മത്സ്യത്തിൻറെ ആവാസവ്യവസ്ഥയിലെ നിലവിലെ നിരോധനം പുനഃപരിശോധനക്ക് വിധേയമാക്കി 'യഥോചിത കാലിക മത്സ്യബന്ധനനിരോധനം' നടപ്പാക്കാൻ ഗവേഷകർ ശുപാർശ ചെയ്യുന്നു.