

Reproductive biology, histomorphometry and annual maturation cycle of *Barilius vagra* in Ladhia river, a mid-altitudinal Himalayan lotic ecosystem of Uttarakhand, India

R. Riyaz¹, P. Dash³, K. Kunal², R. Tandel³, P.B. Sawant¹ and D. Sarma^{1*} 

¹Division of Aquaculture, Indian Council of Agricultural Research-Central Institute of Fisheries Education, Versova, Mumbai-400 061, India

²Indian Council of Agricultural Research- Directorate of Coldwater Fisheries Research, Bhimtal-263 136, India

³Indian Council of Agricultural Research- Central Institute of Brackishwater Aquaculture, Navsari-396 450, India

Received: 03 July 2024

Revised: 20 August 2024

Accepted: 11 November 2024

*Corresponding Author Email: debajit@cife.edu.in

*ORCID: <https://orcid.org/0000-0002-2425-7017>

Abstract

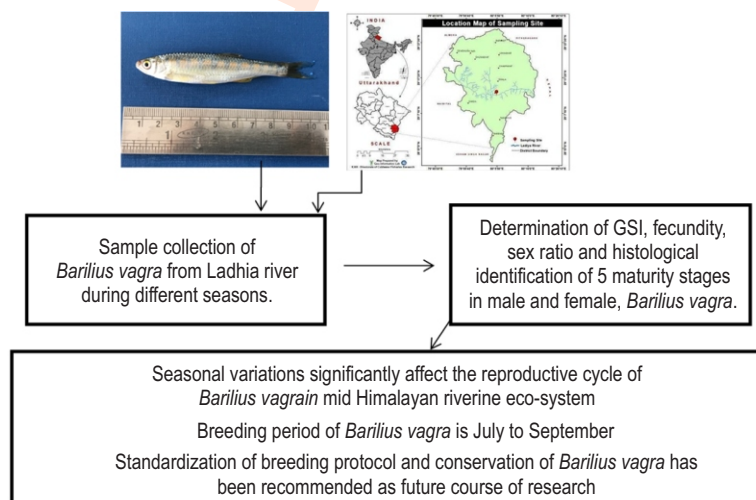
Aim: To assess the influence of seasonal changes on the reproductive indices and gonadal maturation of *Barilius vagra* for future management planning in Central Himalayan rivers.

Methodology: A total of 104 specimens (37 males, 67 females), measuring 5-12 cm and weighing 3-17 g, were collected from the Ladhia River, Uttarakhand, between September 2019 and July 2020. Variations in gonadal histology and gonadosomatic index (GSI) were analyzed to assess reproductive status. Other factors, such as sex ratio, size distribution, condition factor, sexual maturity, fecundity and ova diameter were also studied.

Results: The sex ratio favored females (1:1.12), which were generally larger (8-10 cm). Lm₅₀ for males was 6.6 cm and 7.6 cm for females. Fecundity ranged from 1,640 to 3,354 eggs (9.25±0.2 cm, 5.46±0.31 g), and ova diameters from 17 to 887 µm. The condition factor was less than 1 across seasons, indicating poor well-being of *Barilius vagra*. Gonadal histology revealed five stages of development: spawning capable, regressing, regenerating, developing and immature.

Interpretation: *B. vagra* is an asynchronous spawner with a spawning period from late monsoon to early autumn. Seasonal variations significantly affect its reproductive biology, influenced by environmental factors in the Ladhia River.

Key words: *Barilius vagra*, Fecundity, Gonadosomatic index, Maturity, Oocyte frequency, Sex ratio



Introduction

Ladhia river, a mid-altitudinal lotic Himalayan ecosystem, located in the Champawat district of Uttarakhand, India, is a dynamic and ecologically significant environment, the region's climate characterized by distinct seasonal patterns, impacts the river ecosystem (Bhat *et al.*, 2012). Seasonal changes in temperature, precipitation, and flow rates affect the biological and ecological dynamics of the river. The flow of the river varies seasonally, with the monsoon season bringing increased water levels and flow rates. These hydrological changes influence the river's ecosystem, affecting fish migration, breeding, and other ecological processes (Bhat *et al.*, 2012). Ladhia river is not only important for local biodiversity but also contributes to the larger ecological network of the region (Kumar and Singh, 2018). It also plays a significant role in the broader Himalayan ecosystem. It not only supports the aquatic life but also terrestrial species that depend on the river for water and food. The river's health is integral to the ecological stability of the region as it influences the downstream ecosystems and the overall environmental stability of the area (Sharma *et al.*, 2020).

The river ecosystem faces threats from overfishing, deforestation, pollution and climate change. These challenges require coordinated conservation efforts, including habitat restoration, pollution control, and sustainable resource management (Singh and Lakra, 2011). The fisheries of Ladhia river are characterized by a diverse range of small indigenous fishes (SIF), which play a crucial role in the sustenance and economy of the rural population. Ladhia river is home to various fish species, including *Barilius vagra*, which are an integral part to the local biodiversity and fisheries. Other notable species include mahseer (*Tor sp.*), snow trout (*Schizothorax sp.*) and other cyprinids (Singh *et al.*, 1987; Uniyal and Uniyal, 2021). *Barilius vagra* belongs to bariline group of the family Cyprinidae and sub-family Danionidae and represents an important small-scale indigenous fishery of the Indian hill regions. In general, the fish is one of the valuable ornamental fish exported from India (Jayalal and Ramachandran, 2012) and consumed by the local people in its distributional ranges, where it holds a market value of Rs. 200 to 300 per kg. *B. vagra* is listed under the "least concern" category in India though it is considered as an endangered species in countries like Bangladesh (Bangladesh, 2015).

As per the Records of Zoological Survey of India, the species status is 'Vulnerable' in the state of Uttarakhand, India (ZSI, 2007). *Barilius sp.* along with other sentinel spp. such as *Schizothorax*, *Tor*, *Neolissochilus* migrate downstream in winter and upstream in summer for feeding and reproduction (Kaushik and Bordoloi, 2016). *Bariline* fishes are the supplementary source of income and constitute a vital source of diet for the rural Himalayan household (Chanda, 2017; Kumar and Singh, 2019). These species have a cosmic potential to be integrated into the freshwater aquaculture system (Chanda, 2017) owing to their huge local demand for these indigenous fishes. Also, the exploitation of wild stocks for the ornamental fish trade and food

has to be restricted to conserve natural resources, therefore, there is a need to breed the species to expand the trade (Jayalal and Ramachandran, 2012). Pertaining to the paucity of information regarding seasonal reproductive cycle of *B. vagra*, the present study envisaged to generate information regarding the reproductive cycle associated with sexual maturation of *B. vagra*, which is still lacking to date.

Materials and Methods

Barilius vagra samples were collected by cast net from the Ladhia river in Champawat district of Kumaon Hills, (29°11'36"N 80°5'25" E) Uttarakhand, India between September 2019 to July 2020 bimonthly (Fig. 1a, b). The fishing operation was carried out from early morning to evening during the mid-month for three consecutive days; Monsoon (July and September), Autumn (November), Winter (January), Spring (March), Summer (May). Water temperature was recorded during each sampling month for three consecutive days (morning and afternoon) during the collection period (Fig. 2). Sex was determined at the sampling site by dissecting individual fish. Prior to dissection, the fish were anesthetized with clove oil, and the length and weight of each fish was recorded. The gonads were carefully removed and weighed on a digital scale balance of sensitivity 0.01g. The ovaries and testes were preserved in Bouin's fluid and 10% NBF (Neutral buffer formalin) solution for histological analysis.

Sex was determined after dissection of individual fish. Since identification of sex of each individual was difficult before the developing stage, histological analysis was performed for the confirmation of sex. The size distribution of fish was calculated through frequency distribution curve after grouping males and females in a 1cm length class. Mature ovaries in May and July were used for the estimation of fecundity. The ovaries (preserved in Bouin's fluid) of 15 females (9.25±0.2 cm TL, 5.46±0.31 g) were used for the gravimetric sub-samples weighing 0.01 g weight. Three subsamples of each ovary were submerged in Gilson's fluid (Simpson, 1951), shaken vigorously, and left to stand for 48 hr so the hardened ova would liberate easily from the broken ovarian tissue. Eggs were rinsed with distilled water and dispersed into a clean petridish. Clump fany, was gently separated with a needle and counted under a stereomicroscope (Leica S9i, Germany). Fecundity was estimated by the method of Hickling and Rutenberg (1936). Absolute fecundity was estimated using the equation [(weight of the ovary x average number of eggs per subsample)/ weight of sub-sample], and relative fecundity was evaluated in terms of the number of eggs per unit length of the fish.

The length at which the population attained 50% sexual maturity ($L_{m_{50}}$) was estimated following the method of Ellender *et al.* (2012) and Kindler *et al.* (2015) by fitting a logistic curve to the proportion of reproductively active (developing, ripe, spent) fish in 0.5 cm total length (TL) size classes.

Fulton's condition factor (K) was calculated from the length and weight data of individual fish as $K = 100 \times (\text{weight of}$

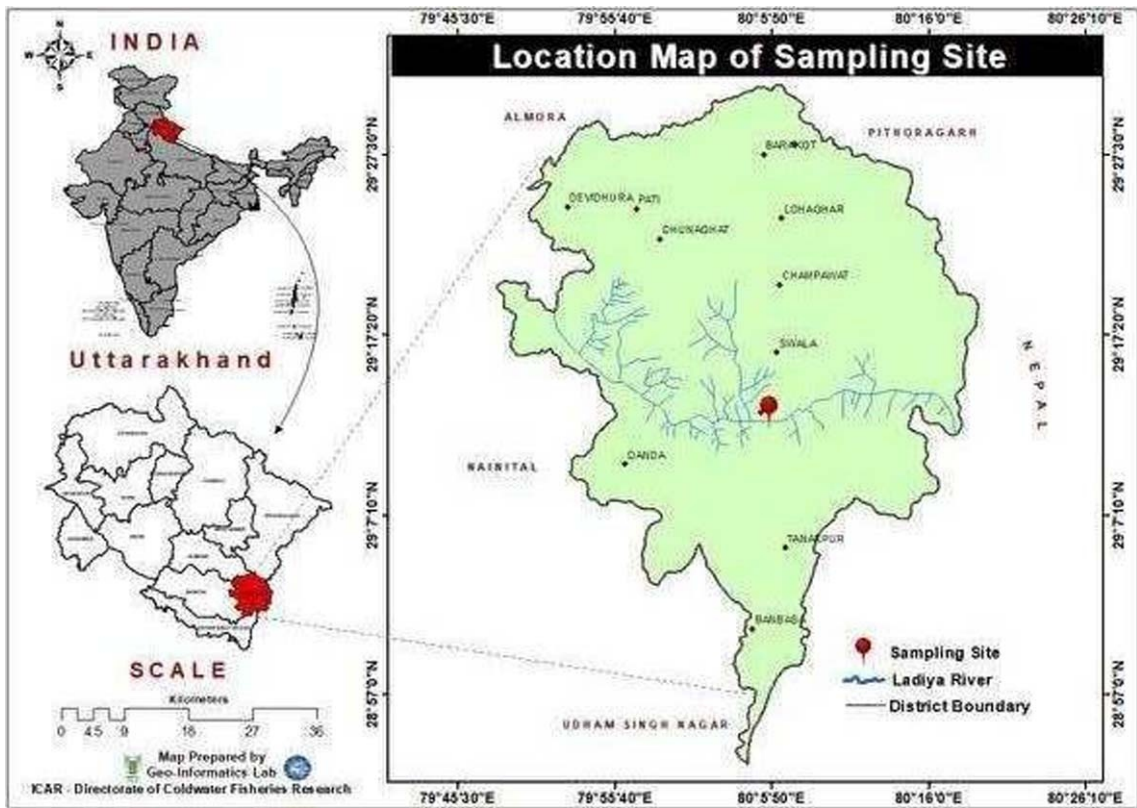


Fig. 1a: Sampling location of *B. vagra* in the Ladhia river during September 2019 and July 2020.

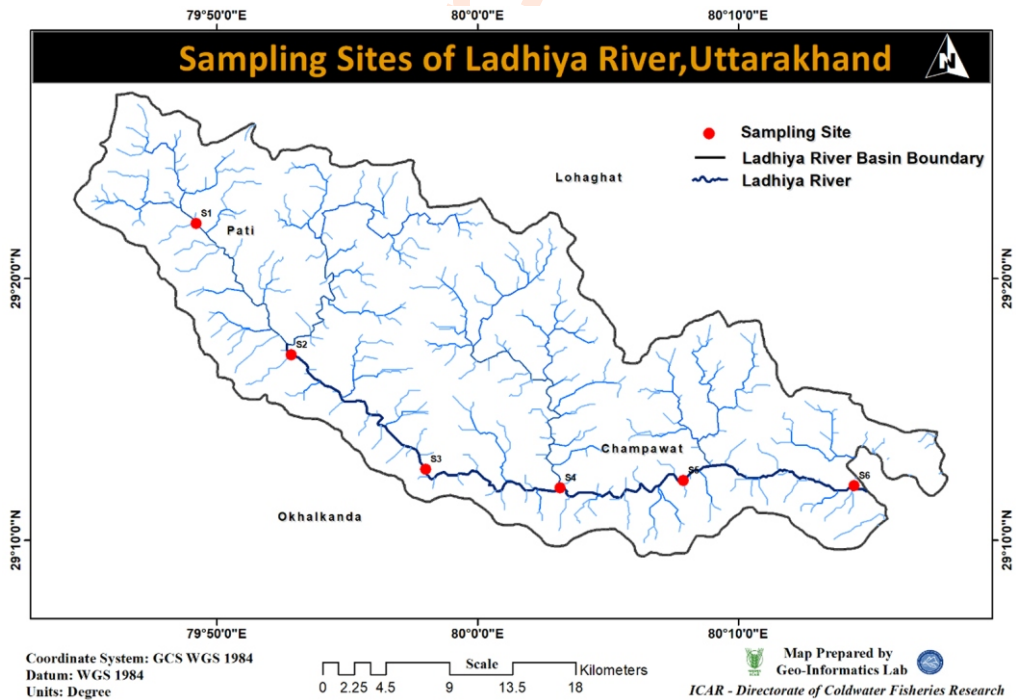


Fig. 1b: GIS based Location of sampling sites of Ladhia river.

fish/length of fish 3). Gonadosomatic index (GSI) was calculated according to Lagler (1956).

The preserved tissues were washed in tap water, placed in cassettes, immersed in 70% ethanol overnight. Thereafter, the tissues were dehydrated in a series of ascending grades of ethanol 70%, 95%, 100% for 45 min. Xylene was used as a clearing solution for removing ethanol from tissues. The tissues were then embedded in wax. Finally, thin sections of tissues (4µm) were cut from the blocks using a microtome, followed by staining the tissues with haematoxylin and eosin. Microscopical staging on all the sections' histological changes was done with a compound microscope (Nikon Eclipse Ci), and representative photomicrographs were taken with a digital camera (Nikon Eclipse Ci). The interpretation of ovarian and testicular developmental phases were described by the terminologies outlined by Peterson *et al.* (2011). Histological photomicrographs were used to check the oocyte stage and size (three slides for each ovary) and eventually assess the seasonal variations in the oocyte stage's distribution percentage following the protocol of Wallace and Selman (1981). Image J software was used to measure the oocyte diameter. Oocyte diameters were measured at the largest diameter (at least 50 for each individual), and stages were noted in each sampling month. The oocyte diameters were measured in image J software from histology slides.

Data were statistically analyzed by statistical package SPSS version 22. Graphs were prepared by Graph Pad Prism 6.0. An Independent t-test was carried out for comparing the mean size (length) between males and females. A Chi-square (χ^2) test was done to compare the proportion of males and females. Kolmogorov-Smirnov test was carried out to compare the size-frequency distribution of males and females. One-way ANOVA and Turkey HSD tests were used to determine the significant differences between the means of condition factor and GSI. Comparisons were made at 95% probability level.

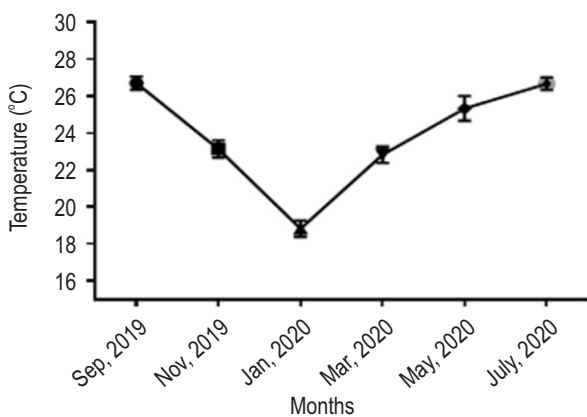


Fig. 2: Bimonthly variations in water temperature at sampling location in the Ladhia river.

Results and Discussion

A total of 104 individuals of *Barilius vagra* with 37 males and 67 females were collected having a TL range of 5-12cm. The size of females ranged between 6-12 cm (9.29±0.17cm), and males 5-10.4 cm (8.12±0.3 cm). The size of males and females were significantly different (t-test, p=0.002). K-S test showed that the length size distribution of males and females were significantly different (D = 0.5, n1 = 67, n2 = 37, p < 0.01). In the 5-8 cm size group, the proportion of males was substantially higher, and greater than 8 cm females were more distributed (Fig. 3).

Significantly more number of females were captured than males ($\chi^2 = 8.64$, df = 1, p = 0.003) with sex ratio of 1.12:1. In September, a significantly higher χ^2 value was observed ($\chi^2 = 18.6$, df = 1, p = 0.00) with the proportion of 92.3% females and 7.7% males (Table 1). The absolute fecundity of fish ranged from 1640-3354 (9.25±0.2cm TL, 5.46±0.31g) and relative fecundity ranged from 186-321 per cm body length. Sex ratio can be influenced by several factors like differential migration of the species according to the environmental condition for feeding and breeding, sex-linked tolerance of species, and sex difference in longevity (Farooq *et al.*, 2019). In the present study, the sex ratio of *B. vagra* showed the dominance of females over males (1.12:1). The highest female population of *B. vagra* was observed in September, which might be attributed to the female's gravid condition, easier to catch. The present study also indicated that *B. vagra* had a similar fecundity compared to other barils (Bahuguna *et al.*, 2021). The length at first maturity (L_m) for males and females was assessed based on a logistic curve as depicted in Fig. 4. In the current investigation, the length at first maturity (L_{m50}) in the case of *B. vagra* males was 6.6 cm and 7.6 cm for females. The smallest mature male sampled during this study measured 6.0 cm and at 7.5 cm length, all males were mature. The smallest mature female fish was 7.0 cm and at 9.5 cm, all females were mature. Length at first maturity should be considered as a minimum size for commercial

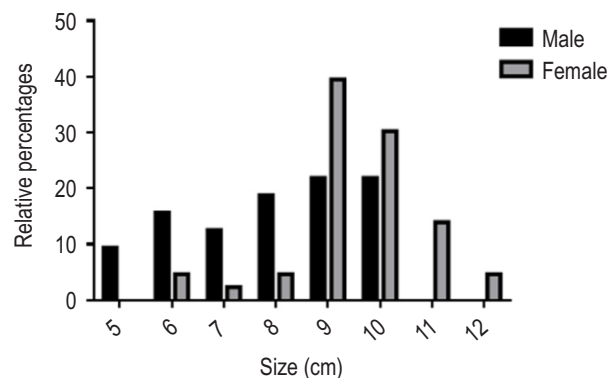


Fig. 3: Size-frequency distribution for females and males of *B. vagra* captured in the Ladhia river between September 2019 and July 2020 (n= 104).

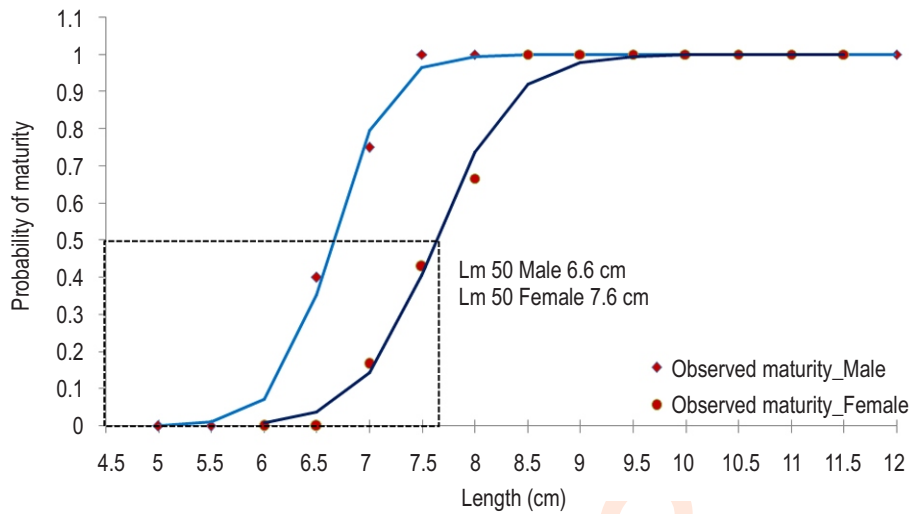


Fig. 4: Adjusted percentages of mature males and females of *B. vagra* versus total length showing the logistic curve fitted to the data collected from Ladhia river, Kumaon Himalaya, India.

Table 1: Monthly variation in sample size and sex ratio (male/female) of *B. vagra*

Months	Total no. of fish	Female (number)	% of females in the sample	Male (number)	% of males in the sample	M:F	χ^2	P value
Sep. 19	26	24	92.30	2	07.69	1:12	18.60	0.00
Nov. 19	23	17	73.91	6	26.08	1:2.83	5.20	0.02
Jan. 20	19	10	52.63	9	47.36	1:1.11	0.05	0.81
Mar. 20	10	4	40.00	6	60.00	1:0.66	0.40	0.52
May. 20	16	8	50.00	8	50.00	1:1.0	0	1.00
Jul. 20	10	4	40.00	6	60.00	1:0.66	0.40	0.52

capture of fish as it would allow the fish to attain certain biomass and spawn at least once in their lifetime (Hasan *et al.*, 2021).

Males often have less energy invested in reproduction compared to females, who usually bear the energetic costs of egg production. Since males don't require more energy for producing large gametes (like eggs), they can mature early. The maturation of males at a smaller size is also found in other small size cyprinids such as *Barbus motebensis* (Kindler *et al.*, 2015) and *Labeo victorianus* (Kembenya *et al.*, 2022). Condition factors for males and females are depicted in Fig. 5. Males and females showed a similar trend in condition factors. A significantly lower value of condition factor was recorded in September ($p < 0.05$) compared to March, May and July. The maximum condition factor for males (0.98 ± 0.04) and females (0.84 ± 0.18) was recorded in March ($p < 0.05$). The condition factor is the compelling morphometric factor to determine the health and physiological status of fish in its natural environment. During September, the condition factor of both males and females was significantly lower, corresponding to the spawning phase of the reproductive cycle. Lower values of condition factor during spawning season

may be related to reproduction as there is a reduction in feeding activity (Sangama *et al.*, 2019). The present findings concur with the studies of Jisr *et al.* (2018), who also reported that during spawning season entire energy is consumed for the development of gonads and spawning, which affects the overall health. The higher value of condition factor during the developing stage indicates fat accumulation and gonadal development (Adeboyejo *et al.*, 2021). Also, the condition factor of fish should be greater than 1 for the well-being of fish in a particular habitat. Since the condition factor during all seasons was less than 1, it suggests that the well-being of fish is threatened in its habitat.

The GSI values of males and females during different seasons are depicted in Fig. 6. In males, the GSI during January and March showed a significantly lower value ($p < 0.05$). The maximum value was recorded during May (4.06 ± 0.39) and minimum during March (0.39 ± 0.2). In females, the GSI during winter showed a significantly lower value ($p < 0.05$). The maximum GSI was recorded during May (7.57 ± 1.2) and minimum during November (1.08 ± 0.06). There was a corresponding fluctuation in cyclic variations and GSI of male and

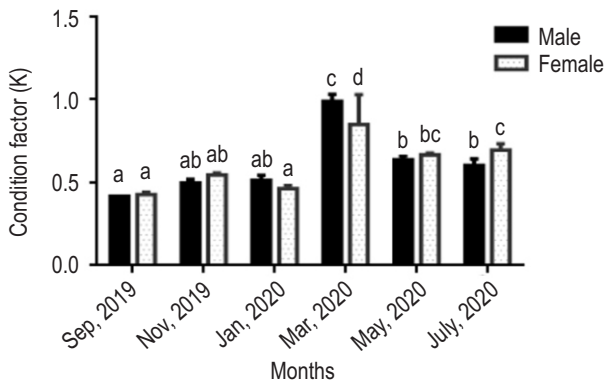


Fig. 5: Seasonal changes in condition factor of males and females *B. vagra*.

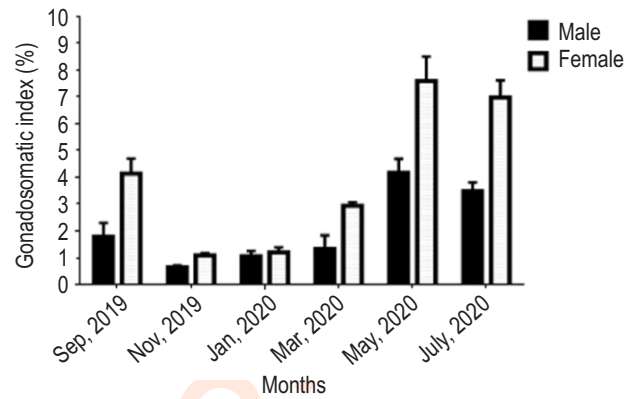


Fig. 6: Seasonal variations in gonadosomatic index of *B. vagra* (n=67 for females and n=37 for males).

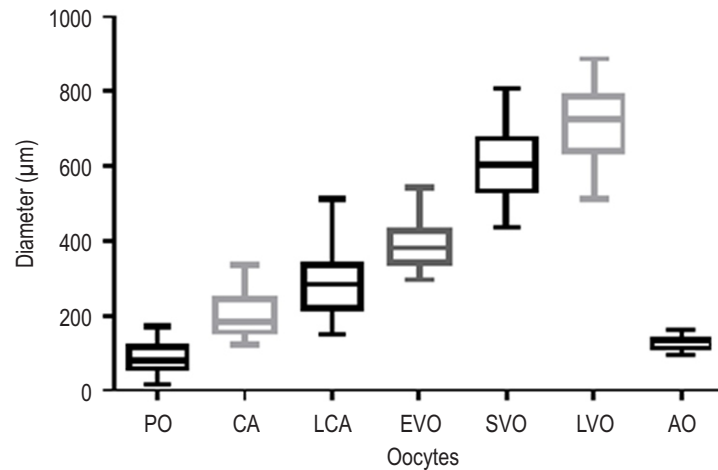


Fig. 7: Size distribution of oocyte's diameter measured by Image J software from histological slides (n=50 for each oocyte). PO: Primary oocyte; CA: Cortical alveolus; LCA: Late cortical alveolus; EVO: Early vitellogenic oocyte; SVO: Secondary vitellogenic oocyte; LVO: Late vitellogenic oocyte; AO: Atretic oocyte.

female gonads of *B. vagra*. The GSI manifested cyclic changes displayed a single highest peak during May, both in the case of males and females. Prior to spawning, fish invest a significant amount of energy for the development of their gonads (testes in males and ovaries in females). This period involves the production and maturation of gametes (sperm and eggs) (Prakash *et al.*, 2022). As a result, the gonads reach their maximum size just before the fish are ready to spawn. The fall in GSI during July and significant fall during September, and appearance of post-ovulatory follicle complexes' indicates that the matured oocytes have already been released during late July and continuing in September. In many teleosts, the presence of post-ovulatory follicle complexes along with the fall in GSI is the accepted indicator of spawning females (Ganias, 2012). Oocyte diameters ranged between 16.8-887.9 µm (Fig. 7). The oocyte size distribution can be used to estimate time to start of spawning (Kjesbu, 1994) and later during spawning, the proportion of eggs spawned (Kjesbu *et al.*, 1990).

Oocyte size distribution can therefore assist in determining the maturity stages in fish and the commencement of spawning.

A macroscopic description of *B. vagra* ovary depicting different phases of reproductive development vis-à-vis spawning capable, regressing, regenerating and developing, along with the percentage frequency of females, is shown in Fig. 8. In September, around 60% females were in regressing phase, with the ovarian histology showing mostly primary oocytes along with post-ovulatory follicles, atretic oocytes, cortical alveolus and early vitellogenic stages (Fig. 8, 10). The remaining 40% females were at the spawning capable stage (including actively spawning) (Fig. 8, 9, 10 a, b). During November and January, more than 60% females were in regenerating phase, with their oocytes observed at an early and late perinucleolus stages along with some atretic oocytes (Fig. 8, 9, 10 c). The progress of secondary oocyte development was observed in March as shown in Fig. 9 and 10 d.

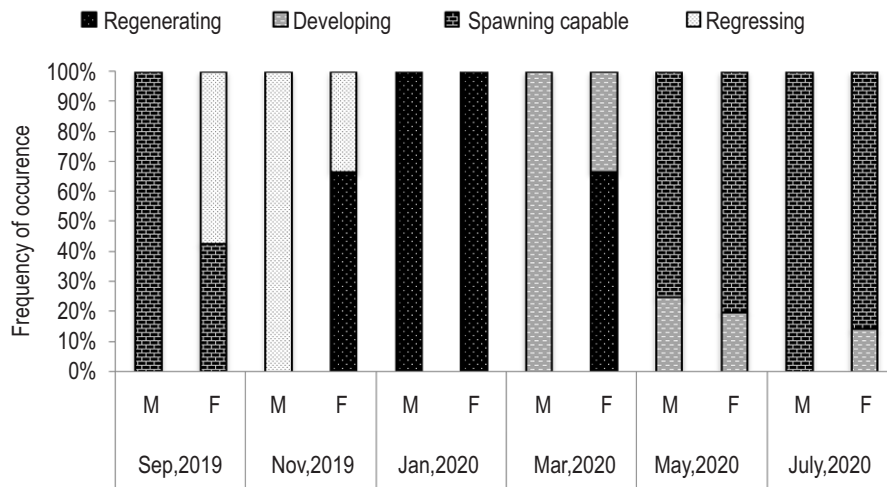


Fig. 8: Percentage frequency occurrence of male and female *B. vagra* in different maturation stages across the sampling periods (minimum n = 6 for males and minimum n=10 females per month).

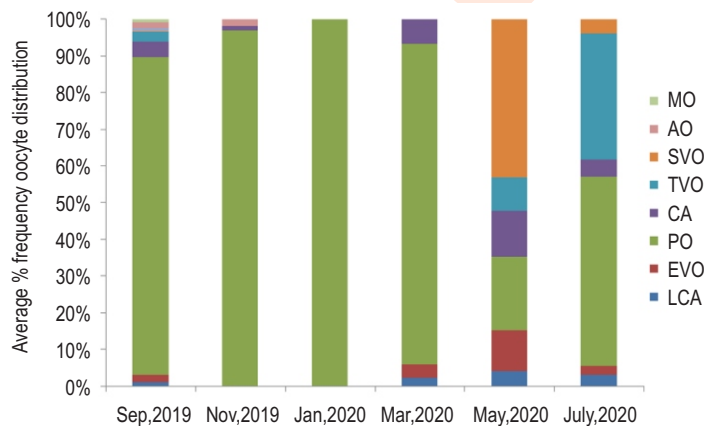


Fig. 9: Seasonal variation in the percentage of oocyte stages in *B. vagra* females (minimum n =10 females per month). The oocyte stages were estimated from the histology slides.

During May and July, more than 80% females were at a spawning capable stage, with their oocytes at both primary and secondary growth phases (Fig. 10 e, f). During this period, Vtg 3 was the most advanced stage of oocyte present in the ovary (Fig. 9).

The histological study of female gonads suggest that stages of oocyte development are identical with other cyprinids such as *Barilius bendelisis*, *Barbus motebensis* and *Puntius tetrazona* (Saxena et al., 2018; Arman and Ucuncu, 2020). *B. vagra* has an extended spawning period, lasting from late monsoon (July) to early autumn (September) unlike *B. bendelisis*, which spawns from May to July (Saxena et al., 2019). The reproductive status of *B. vagra* differs from other co-existing cyprinid, *Schizothorax richardsonii*, while the later species have bimodal reproductive status (Ciji et al., 2021).

The macroscopic description of testes of *B. vagra* depicting different phases of development vis-à-vis spawning capable, regressing, regenerating and developing, along with the percentage frequency of males, is shown in Fig.8. All the sampled males were at spawning capable (including actively spawning) phase during September. Mature testes were characterized by the presence of a higher degree of tubular enlargement, wherein tubular lumens were filled with mature spermatozoa and spermatids. Spermatogonia and spermatocytes were rarely visible, and some empty tubules were observed (Fig. 11 a). All the males were identified in regressing phase with their tubules during November constricted with a thin layer of widely scattered spermatocysts in tubular boundary and residual spermatozoa in the lumen (Fig. 8, 11 b). In January, all the sampled males were in regenerating phase wherein their tubules were highly constricted,

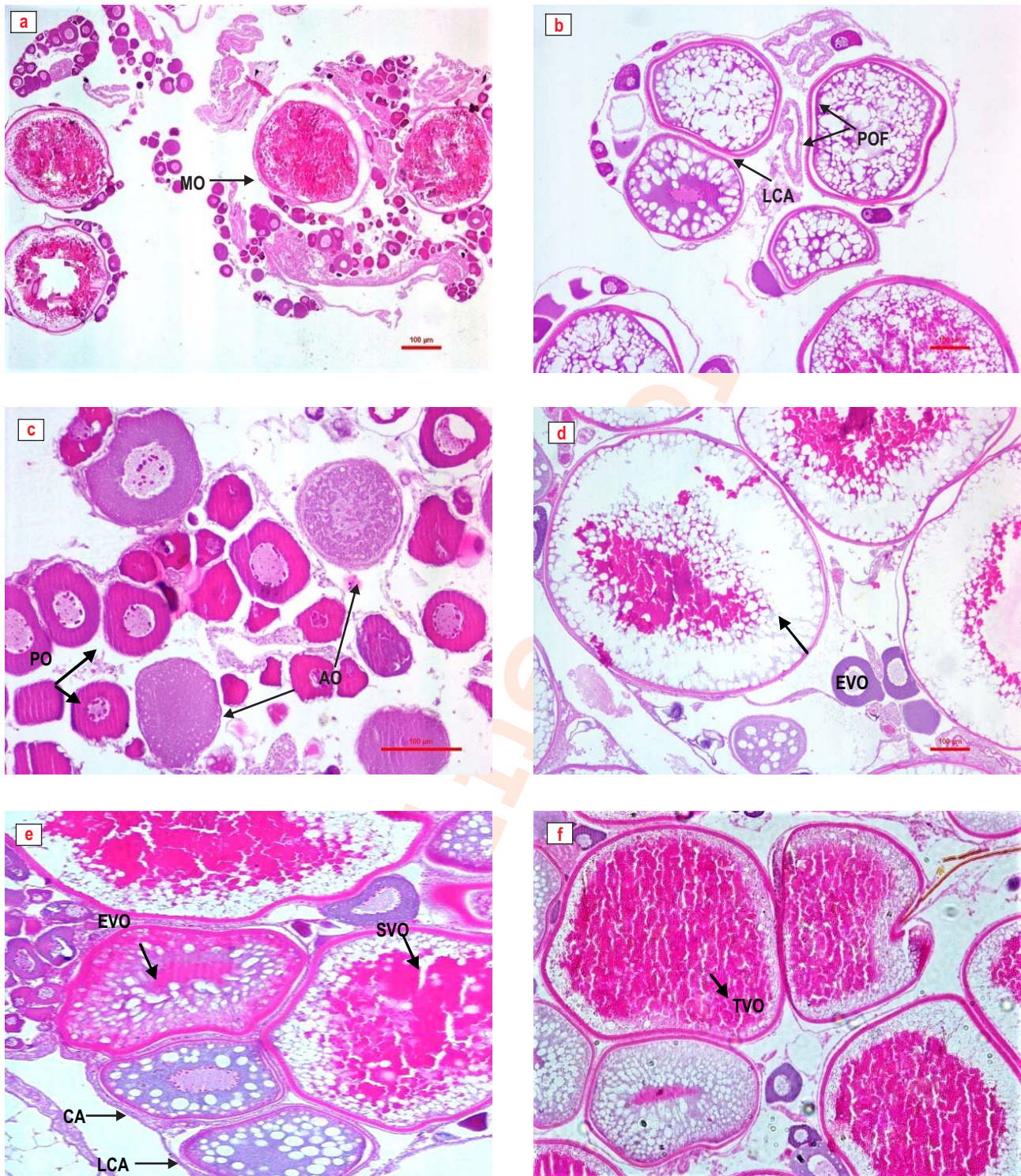


Fig. 10: Histological illustrations of ovary of *B. vagra* sampled during Sep, Nov, Mar, May, July (a): Regressing phase: ovarian section during September showing a mature oocyte (MO), and postovulatory follicle (POF) (b) Regressing phase: ovarian section during September showing postovulatory follicle complexes (POFs), (c) Regenerating phase: ovarian section during November showing follicular atresia (AO) & primary growth oocyte (PO) (d) Developing phase: ovarian section in March showing early vitellogenic oocyte (EVO) (e) Developing phase: ovarian section during May showing cortical alveolus (CA), late cortical alveolus oocyte (LCA), early vitellogenic oocyte (EVO), secondary vitellogenic oocyte (SVO) (f) Spawning capable phase: ovarian section during July showing tertiary vitellogenic oocyte (TVO).

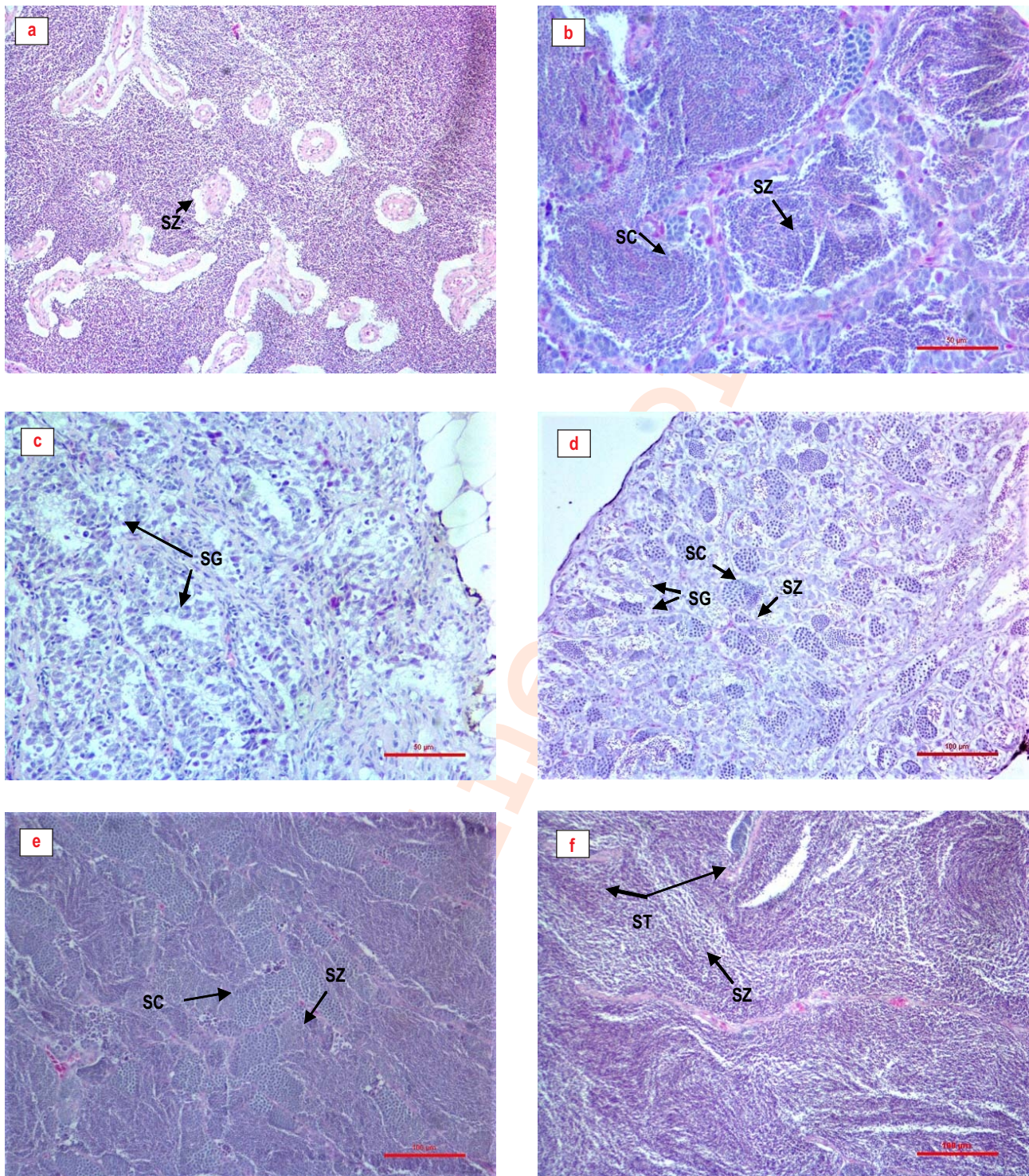


Fig. 11: Photomicrographs of testes sections of *B. vagra* sampled during Sep, Nov, Jan, March, May, July (a) Spawning capable phase: testicular section during September showing spermatozoa (SZ), (b) Regressing phase: testicular section during November showing spermatocytes (SC) and spermatozoa (SZ) (c) Regenerating phase: testicular section during January showing spermatogonia (SG), (d) Developing phase: testicular section during March showing spermatocyst (SC), spermatogonia (SG) and spermatozoa (SZ), (e) Spawning capable phase: testicular section during May showing spermatocysts (SC) and spermatozoa (SZ), (f) Spawning capable phase: testicular section during July showing spermatids (ST) and spermatozoa (SZ).

lined with spermatogonia (SG), few spermatocysts and lumen had no traces of spermatozoa (Fig. 11 c). In March, the testes samples showed the development of spermatogenesis with the presence of clusters of spermatogonia cells, spermatocysts, and the formation of spermatozoa (Fig. 11 d). During May, more than 70% of the males were at spawning capable phase whereas in July, all the males were capable of spawning (Fig. 8, 11 e, f). During this period, tubular lumens were filled with mature spermatozoa with the presence of spermatocysts in spermatocysts.

The microscopic staging and histological features of male gonads followed a similar reproductive pattern as females. The presence of empty tubules along with a fall of GSI in September suggested the spawning of males (Peterson *et al.*, 2011). The breeding phase ceases during November as the spermatozoa are present in the lumen, and the spermatocysts are widely scattered. Further, continuous germinal epithelium and spermatozoa in the spermatocyst and not in the lumen of the lobule suggest that the testes are in the developing/ maturing stage during March (Ciji *et al.*, 2021). Seasonal changes in water temperature are considered a prime regulatory factor for the spawning of many freshwater fishes (Tessema *et al.*, 2020). The observed increasing activity of spermatogenesis, oogenesis, and GSI values of males and females of *B. vagra* between March to September, might be associated with higher water temperature values of 23 to 26°C. Hence, the seasonal reproductive cycle of *B. vagra* would generate baseline information for developing the seed production protocol and would prove to be a considerate study for the protection and conservation of this small indigenous fish throughout its geographical location. Further, this study will also be helpful in conservation and restoration planning in the changing climatic scenario of an important native species inhabiting a lotic mid-altitudinal Himalayan eco-system of Uttarakhand, India.

Acknowledgments

The authors owe their deep sense of thanks to Dr. Neetu Shahi and Mr. Krishna Kala for their kind support in the histology part. The authors also duly acknowledge to Mr. Parvez Ahmed, Mr. Maneesh Dube and Dr. Raghvendra Singh for their assistance in sampling. The present work was carried out under, ICAR.

Authors' contribution: R. Riyaz: Original draft preparation, Methodology, Formal analysis; P. Dash: Conceptualization, Methodology, Original draft preparation, Formal analysis; K. Kunal: Methodology; R. Tandel: Methodology, Reviewing and Editing; P.B. Sawant: Supervision; D. Sarma: Project Administration, Resources & Supervision.

Funding: The study was carried funded under the project All India Network Project on Ornamental Fish Breeding and Culture (NPOFBC) by the Indian Council of Agricultural Research, New Delhi.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: The study complies with the current animal welfare laws in India following the guidelines of the CPCSEA [(Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment & Forests (Animal Welfare Division), Govt. of India] on care and use of animals in scientific research. The Institutional Animal Ethics Committee, corresponding author's affiliation, ICAR-DCFR ref. No. DCFR/IACUC/23/07/2021/1 approved the protocols for handling and sampling fish during experiments.

Conflict of interest: The authors declare that there is no conflict of interest.

Data availability: The supplement data generated and analyzed during the current study are available from the corresponding author on reasonable request.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology*.

References

- Arman, S. and S.I. Ucuncu: Gonadal histology of the tiger barb *Puntius tetrazona* (Cyprinidae). *J. Fish.*, **8**, 817-822 (2020).
- Simpson, A.C.: The Fecundity of Plaice. UK Ministry of Agriculture and Fisheries, Fisheries and Food Investigation., **217**, 3-27 (1951).
- Adeboyejo, O.A., E.O. Clarke, A.M. Hammed, O.O. Whenu, J.P. Abayomi and O.M. Olarewaju: Abundance and distribution, growth pattern, sex ratio and gonadosomatic index (GSI) of *Liza falcipinnis* (Valenciennes, 1836) from Ojo Axis of Badagry Creeks, Lagos, Nigeria. *Sustain. Mar. Strc.*, **3**, 39-49 (2021).
- Singh, H.R., S.P. Badola and A.K. Dobriyal: Geographical distributional list of ichthyofauna of the Garhwal Himalaya with some new records. *J. Bom. Nat. Hist. Soc.*, **84**, 126-132 (1987).
- Bahuguna, P., R. Kumar and K. Shah: Breeding power and sex-ratio in *Barilius vagra* (Ham.) from spring-fed river Mandal, Garhwal Himalaya, India. *Aquaculture*, **10**, 279-283 (2009).
- Bahuguna, P., Sapna, R. Rayal, N. Shah and N.C. Khanduri: Sexual maturity of an ornamental Himalayan foot hill region fish *Barilius bama* as determined by Dobriyal index and gonadosomatic index. *Scient. Tempe. R.*, **XI**, 75-80 (2020).
- Bangladesh, I.: Red List of Bangladesh: Freshwater Fishes. IUCN, International Union for Conservation of Nature, Bangladesh Country Office, Dhaka, *Bangladesh*, **5**, 360 (2015).
- Barman, R.: A review of the fresh waterfish fauna of West Bengal, India with suggestions for conservation of the threatened and endemic species. *Records of the Zoological Survey of India, Occasional Papers*, **263**, 1-48 (2007).
- Bhatt, J.P., K. Manish and M.K. Pandit: Elevational gradients in fish diversity in the Himalaya: water discharge is the key driver of distribution patterns. *PLoS ONE.*, **7**, e46237 (2012).
- Chanda, A.: Record of two threatened fish species under genus *Barilius* Hamilton, 1822 from Paschim Medinipur District of West Bengal. *WWJ Multi Res Dev.*, **3**, 79-83 (2017).
- Ciji, A., P. Sharma, M. Rajesh, B.S. Kamalam, A. Sharma, P. Dash and M. Akhtar: Intra-annual changes in reproductive indices of male and female Himalayan snow trout, *Schizothorax richardsonii* (Gray, 1832). *Aqua. Res.*, **52**, 130-141 (2021).
- Dey, A., R. Nur, D. Sarkar and S. Barat: Captive breeding through synthetic hormone induced breeding of an eastern Himalayan hill

- stream fish, *Barilius barila* (Hamilton, 1822). *Int. J. Fish. Aqua. Stud.*, **4**, 354-358 (2016).
- Ellender, B., O. Weyl and H. Winker: Age and growth and maturity of southern Africa's largest cyprinid fish, the largemouth yellowfish *Labeobarbus kimberleyensis*. *J. Fish. Bio.*, **81**, 1271-1284 (2012).
- Farooq, I., F. Bhat, M. Balkhi, T. Shah, B. Bhat, S. Qadri, S. Talia, S. Aalia and P. Ganie: Reproductive and breeding biology of *Schizothorax labiatus*, a snow trout found in River Jhelum, Kashmir. *J. Env. Bio.*, **40**, 291-294 (2019).
- Ganias, K.: Thirty years of using the postovulatory follicles method: overview, problems and alternatives. *Fish. Res.*, **117**, 63-74 (2012).
- Halwart, M.: Biodiversity, nutrition and livelihoods in aquatic rice-based ecosystems. *Biodiversity*, **9**, 36-40 (2008).
- Hasan, M.R., M.Y. Hossain, Z. Mawa, S. Tanjin, M.A. Rahman, U.K. Sarkar and J. Ohtomi: Evaluating the size at sexual maturity for 20 fish species (*Actinopterygii*) in wetland (Gajner Beel) ecosystem, north-western Bangladesh through multi-model approach: A key for sound management. *Acta. Ichth. Et. Piscatoria*, **51**, 29-36 (2021).
- Hickling, C. and E. Rutenberg: The ovary as an indicator of the spawning period in fishes. *J. Mar. Biol. Assoc. UK.*, **21**, 311-317 (1986).
- Jan, M. and I. Ahmed: Assessment of fecundity, gonadosomatic index and hepatosomatic index of snow trout, *Schizothorax plagiostomus* in river Lidder, from Kashmir Himalaya, India. *Int. J. Fish. Aqua. Stud.*, **4**, 370-375 (2016).
- Jayalal, L. and A. Ramachandran: Export trend of Indian ornamental fish industry. *Agri. Biol. J. Nor. Amer.*, **3**, 439-451 (2012).
- Jisr, N., G. Younes, C. Sukhn and M.H. El-Dakdouki: Length-weight relationships and relative condition factor of fish inhabiting the marine area of the Eastern Mediterranean city, Tripoli-Lebanon. *Egypt. J. Aqua. Res.*, **44**, 299-305 (2018).
- Juntti, S.A. and R.D. Fernald: Timing reproduction in teleost fish: Cues and mechanisms. *Curr. Opi. Neurobiol.*, **38**, 57-62 (2016).
- Kaushik, G. and S. Bordoloi: Ichthyofauna of Ranganadi River in Lakhimpur, Assam, India. *Check List*, **12**, 1872 (2016).
- Kembenya, E., A. Getabu, N. Njiru and R. Omondi: Some aspects of the reproductive biology of *Labeo victorianus* (Boulenger, 1901) from Kuja-Migori River basin, Kenya. *J. Fish.*, **10**, 101205 (2022).
- Kindler, D., G. Wagenaar and O. Weyl: An assessment of the reproductive biology of the Marico barb *Barbus motebensis* from the upper Groot Marico catchment, South Africa. *Afri. J. Aqua. Sci.*, **40**, 425-431 (2015).
- Kjesbu, O.S.: The spawning activity of cod, *Gadus morhua* L. *J. Fish. Biol.*, **34**, 195-206 (1989).
- Kjesbu, O.S.: Time of start of spawning in Atlantic cod (*Gadus morhua*) females in relation to vitellogenic oocyte diameter, temperature, fish length and condition. *J. Fish. Biol.*, **45**, 719-735 (1994).
- Kumar, A. and R. Singh: Biodiversity and ecological significance of the Ladhia River in Uttarakhand. *J. Environ. Mangmnt.*, **217**, 509-517 (2018).
- Kumar, S. and D. Singh: Genetic and morphometric comparison of two isolated populations of *Barilius bendelisis* (Cypriniformes: Cyprinidae) from the Indian Himalayas. *Revista de Biol. Tropical.*, **67**, 466-477 (2019).
- Lagler, K.F.: Freshwater Fishery Biology. 2nd Edn., W.C. Brown Co., Dobuque, Iowa, USA (1956).
- Mahapatra, B., K. Vinod and W.S. Lakra: Biology of the ornamental cyprinid fish *Danio dangila* (Hamilton, 1822) from the North-eastern hill region of India. *Ind. J. Fish.*, **63**, 122-125 (2016).
- Panda, A. and M.K. Datta: Length-weight relationship and relative condition factor of *Barilius bendelisis* (Hamilton, 1807) and *Barilius barna* (Hamilton, 1822) from Manu River, Tripura, India. *J. Ent. Zool. Stud.*, **5**, 2535-2539 (2017).
- Patiyal, S., R. and J.I. Mir: Length-weight relationships of 21 fish species from the upland Ganga River Basin tributaries of Central Indian Himalaya. *J. App. Ichth.*, **33**, 861-863 (2017).
- Peterson, N., D. Wyanski, F.S. Rey, B. Macewicz and S. Barbieri: A standardized terminology for describing reproductive development in fishes. *Marine and Coastal Fisheries: Dynamics. Mangm. Ecosystm. Sci.*, **3**, 52-70 (2011).
- Prakash, S.: Condition factor, Hepato-somatic index and Gonadosomatic index of Fish, *Channa punctatus* collected from Sawan Nallaha, Balrampur, UP. *Scient. Temper.*, **13**, 46-50 (2022).
- Raagam, P. and K.R. Devi: An overview of the hill trouts (*Barilius* spp.) of the Indian region. *Zoos' Print J.*, **20**, 1847-1849 (2005).
- Roos, N., M.A. Wahab, C. Chamnan and S.H. Thilsted: The role of fish in food-based strategies to combat vitamin A and mineral deficiencies in developing countries. *J. Nutrtn.*, **137**, 1106-1109 (2007).
- Sangma, S.K., P. Bhattacharjee and P. Pal: Length-weight relationship, Relative length of gut and gastrosomatic index of *Chanda nama* (Hamilton, 1822) and *Trichogaster lalius* (Hamilton, 1822) from Tripura, India. *J. Entomol. Zool. Stud.*, **7**, 737-742 (2019).
- Saxena, N., R.S. Patiyl, K. Dube and V.K. Tiwari: Ovarian maturation and histological observations of *Barilius bendelisis* (Hamilton) in captivity. *Ind. J. Animl. Res.*, **52**, 695-701 (2018).
- Saxena, N., R.S. Patiyl, K. Dube, V.K. Tiwari and J.I. Mir: First attempt of captive breeding, embryonic and larval development of *Barilius bendelisis* (Hamilton 1807). *Ind. J. Animl. Res.*, **53**, 281-287 (2019).
- Sharma, P., A. Joshi and D. Bhatt: Role of Himalayan rivers in sustaining regional biodiversity: A case study of the Ladhia River. *Ind. J. Ecol.*, **47**, 274-283 (2020).
- Sharma, V., A. Srivastava and V.K. Vashistha: The effect of ultraviolet light exposure on proximate composition, amino acid, fatty acid, and micronutrients of coldwater fish, "*Barilius vagra*". *Orientl. J. Chem.*, **35**, 1220 (2019).
- Shimizu, A.: Effect of photoperiod and temperature on gonadal activity and plasma steroid levels in a reared strain of the mummichog (*Fundulus heteroclitus*) during different phases of its annual reproductive cycle. *Gen. Comp. Endocrin.*, **131**, 310-324 (2003).
- Singh, A.K. and W.S. Lakra: Risk and benefit assessment of alien fish species of the aquaculture and aquarium trade into India. *Rev. Aquacul.*, **3**, 3-18 (2011).
- Singh, D. and S. Kumar: Phenotypic divergence and molecular characterization of two sympatric species of genus *Barilius* (Hamilton) from the River Chenab of Western Himalaya. *J. App. Ichth.*, **35**, 954-962 (2019).
- Spence, R., G. Gerlach, C. Lawrence and C. Smith: The behaviour and ecology of the zebrafish, *Danio rerio*. *Biol. Reviews.*, **83**, 13-34 (2008).
- Tessema, A., A. Getahun, S. Mengistou, T. Fetahiand E. Dejen: Reproductive biology of common carp (*Cyprinus carpio* Linnaeus, 1758) in Lake Hayq, Ethiopia. *Fish. Aqua. Sci.*, **23**, 1-10 (2020).
- Uniyal, D.P. and M. Uniyal: Fish fauna of Uttarakhand, India: Present status, diversity and conservation. *J. Mountain Res.*, **16**, 301-324 (2021).
- Wallace, R.A. and K. Selman: Cellular and dynamic aspects of oocyte growth in teleosts. *Am. Zoolog.*, **21**, 325-343 (1981).
- ZSI.: Faunal Diversity Western Doon Shiwalics. Zoological Survey of India, Kolkata, India, pp. 1-133 (2007).