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Circannual Behavioral Study in Indian Hill Trout Fish *Barilius bendelisis* (Hamilton)

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Abstract: In the present study, we collected the fishes from the lake throughout the year for their behavioral study with the help of Ethovision XT 13 (Noldus, Infotech, Wageningen, the Netherlands). Different behavioral parameters such as mobility, turn angle, meander, and area of exploration were recorded by using the Basler camera and software. The behavioral profile of each fish was scored for 300 sec at the rate of 25 frame/sec. The digitized scored data was analyzed by using the Graph pad prism. All the parameters showed a highly significant difference at $P<0.05$.

Keywords: Behavioral, Ethovision, Turn angle, Meander, Indian hill trout, *Barilius bendelisis*

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Introduction

Barilius bendelisis is included in the Cyprinidae family and is benthopelagic in nature. It is a freshwater teleost fish commonly known as Indian hill trout (Dewan and Singh, 2016; Sharma *et al*., 2017). It is an ornamental fish used for food purposes ((Ansuman Panda and Mrinal Kanti, 2017).

It is found in Southeast Asia (Himalayan regions), mainly in Nepal, Myanmar, Bangladesh, Bhutan, India, Sri Lanka, and Pakistan (Sharma *et al*., 2015). Locally it is recognized by various names such as Jhojha, Buddhi, Chadu. From Asia's Himalayan and sub-Himalayan regions, about 20 species of the genus *Barilius* have been recorded (Dewan *et al.*, 2016). It lives in cold-water rivers and streams with gritty and rocky surfaces, these rivers and streams are situated along the base of hills, and this fish can also easily live in lotic and lentic water systems (Dewan *et al.*, 2015). *Barilius bendelisis* possesses a flattened body, bars present

on the body which are blackish-blue in color, and a dorsal fin present behind the middle of the body (Panda *et al*., 2019). Sexual dimorphism was described in *Barilius bendelisis*. Compared to female fish, male fishes are larger in size, bright in color, having rough bodies with mini tubercles on the head, lips and dorsolateral scales on the body (Sharma *et al.*, 2017).

Habituation is the most accessible form of learning, and it is used for behavioral research of animals which helps to investigate the cognitive phenotype of animals. It is found that when animals become habitual to their environment, depletion of innate behaviors occurs. Fishes can also make cognitive maps and spatial memories (Wong *et al.* 2010). Generally, fish are nurtured and grow in stable, predator-free ponds, which are available with prepared food, due to which fishes cannot learn to survive in their domesticated environments. On the other hand, wild fishes in their environments are surrounded by a more complex and diverse habitat, which helps them to learn from experiences and survive in harsh conditions (Ullah *et al.,* 2017). It is found that a nurturing environment increases the adaptability of behavior and feed-finding ability. Enhancement of social and physical things in a foster environment can affect the necessary skills of life, such as catching natural prey for food, escaping from predators, and exploratory behavior (Ullah *et al*., 2017). Fosterage environment with more complications can cause the development of cognition, fish brain, courage, and survival in the wild environment.

For behavioral study, the open field test traditionally used for Rodents is also used as new research in zebrafish. When zebrafish enter the open field arena, it stays near the walls and corners. As it adapts to the environment, it comes to the central area of the arena and increases its area exploration (Stewart *et al.*, 2010). Behavior study of Zebra fish shows that when fish is under stress, it shows freezing, decreased time in the upper zone, more erratic movement, and takes longer latency to arrive upper half zone (Egan *et al.*, 2009). The current investigation investigated the circannual behavioral aspects of *Barilius bendelensis*. There are no records of the prior work on the circannual behavioral aspect of Indian hill stream trout.

Materials and Methods

Collection of fish:

Fish samples were collected from a stream in Suwalekh village (29.66°N, 80'.22E) of district Pithoragarh, India. This stream is a branch of Eastern Ramganga. Throughout the year (January 2021 to December 2021), adults of *Barilius bendelisis* were obtained from the Suwalekh stream with the help of local fishers. Those fishes were selected for the study which seemed healthy and infection free. Subsequently, captured fishes were transported to the laboratory of the Zoology Department of L.S.M. Govt. P.G. College, Pithoragarh (Uttarakhand) in a 10 L container. In the laboratory, fish were kept in tanks (61×61×32 cm) with a constant air supply through the aerator. Fish were acclimatized for seven days to minimize the handling stress. During acclimatization, fish were fed with *ad libitum* artificial food twice daily (crude protein 32%, crude fiber 5%, crude ash 10%, crude fat 4%, Nitrogen 31%, moisture 9%). 70% of water was changed every three days, and the excretory debris was removed. Throughout the year, 60 fish were used for behavioral analysis, i.e. five fish per month. All the ethical guidelines of the national standard were followed for the fish study.

Behavioral study:

After acclimatization, five active fish were randomly selected for behavioral study. Before behavior testing, a rectangular tank (50×30×30 cm) was selected and filled with 50% water. It provided air supply through the aerator for 15-30 min to maintain the oxygen level in the tank before the start of the behavioral observational study. Proper light facilities were provided to the room where behavioral videos were recorded to minimize the artifacts. During the video recording, the aerator was also removed to minimize the

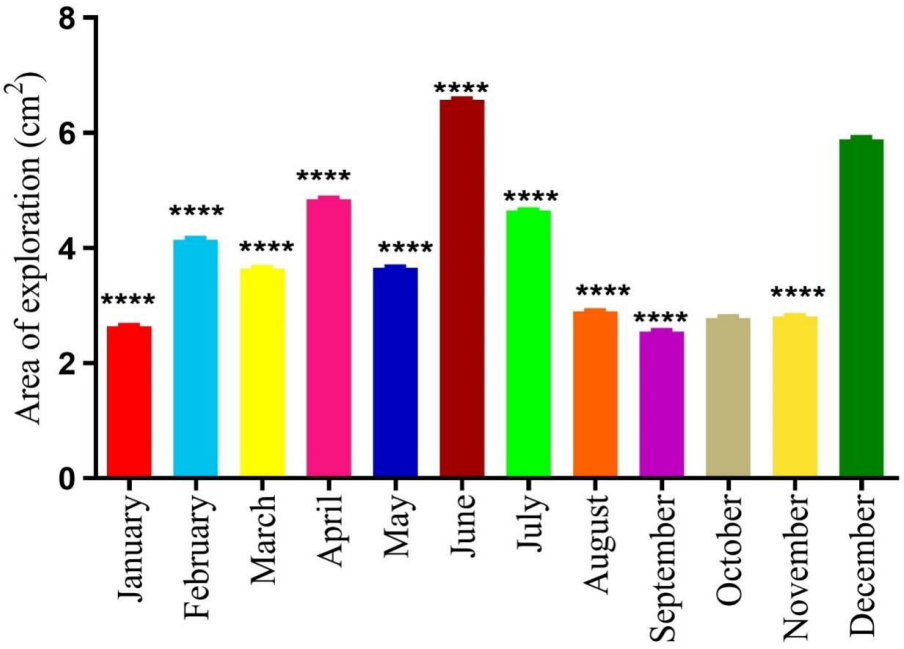

Fig.1: Area of exploration by *Barilius bendelisis*. **** indicates P<0.0001.

artifact of aerator noise. An open field tank was used for behavior testing, and video was recorded with a digital Basler Camera with a pylon four-camera for 300 sec at the rate of 25 frames/sec. The camera was positioned at the top view and connected to the computer control, and then videos were acquired in video-tracking Ethovision XT-13 software (Noldus, Wageningen, Holland). This software evaluated all phenotypic movements of fish and converts them into numerical data. Different kinds of movements of fishes such as Turn angle, meander, the total area explored, and mobility were studied. Mobility included Mobility %, highly mobile, mobile, and immobile states.

Statistical analysis:

Statistical analysis of behavioral data was analyzed using Graph pad prism version 8. A one-way ANOVA analysis with the respective post hoc test was used for the statistical differences.

Results

Area of exploration:

In the circannual behavioral study of fish *Barilius bendelisis*, the area of exploration was maximum in June (6.57 ± 0.003 cm^2) and minimum in September (2.55 ± 0.003 cm^2). One-way ANOVA (F (11, 76463) = 26800; P<0.0001) followed by Tukey's multiple comparison test exhibited noteworthy changes among the months of the year (P <0.05) (Fig. 1).

Turn angle:

During the behavior study,the Turn angle was recorded at maximum in April (105.7 ± 0.3587 Deg) and minimum in August (26.32 ± 0.197 Deg). One-way ANOVA (F (11, 76469) = 5255) followed by Bonferroni's multiple comparison test showed a significant change (P<0.0001) among the consecutive months of the year (Fig. 2).

Meander:

All data were analyzed through one-way ANOVA (F (11, 76469) = 310.1; P<0.0001) followed by Bonferroni's multiple comparison test, which displayed a noteworthy change between meander of entire months. The average meander was

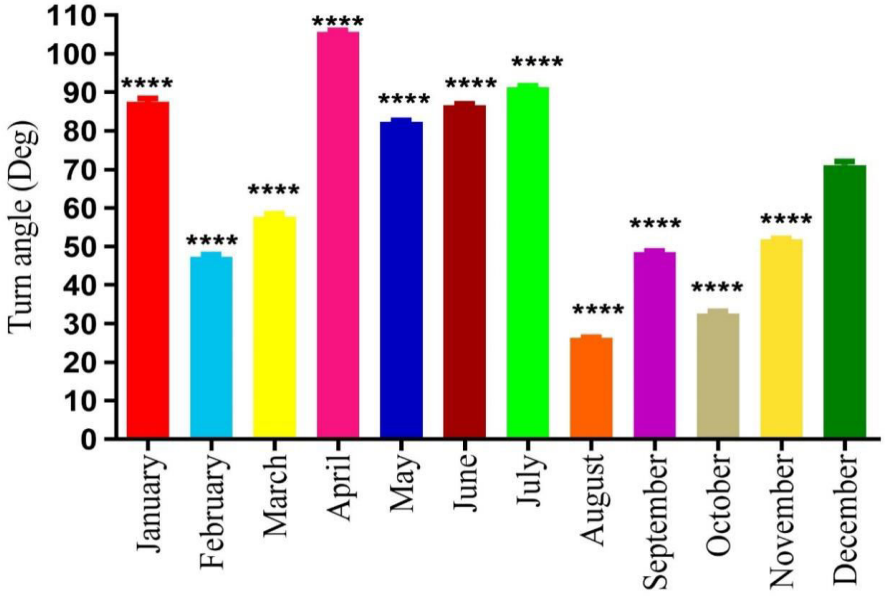

Fig. 2: Turn angle of fish *Barilius bendelisis* during the Circannual behavior study. **** indicates P<0.0001.

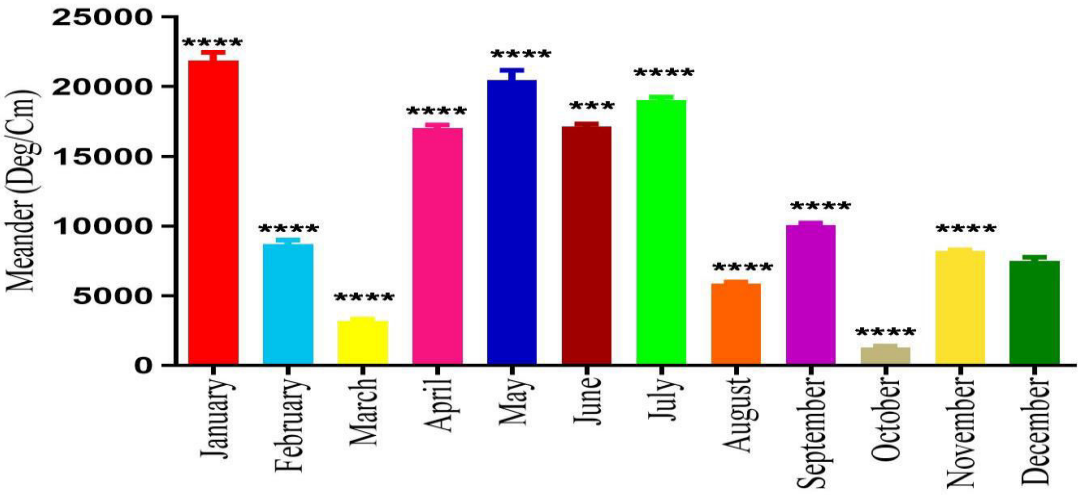

Fig. 3: Meander of *Barilius bendelisis* during the behavioral observation tank. *** and**** indicate P<0.001 and P<0.0001, respectively.

highest in January (21881 ± 570.7 deg/cm) and lowest in October (1298 ± 90.18 deg/cm) during the circannual behavior study (Fig. 3).

Mobility (*%*):

During Behavior profiling of *B. bendelisis,* average mobility was highest in October (39.06 ± 0.42 %) and lowest in June (2.912 ± 0.013 %). Data were analyzed by One-way ANOVA (F (11, 76469) = 8418; P<0.0001) followed by Bonferroni's multiple comparison test which presented a noteworthy change (P<0.05) between the mobility of all the months (Fig. 4).

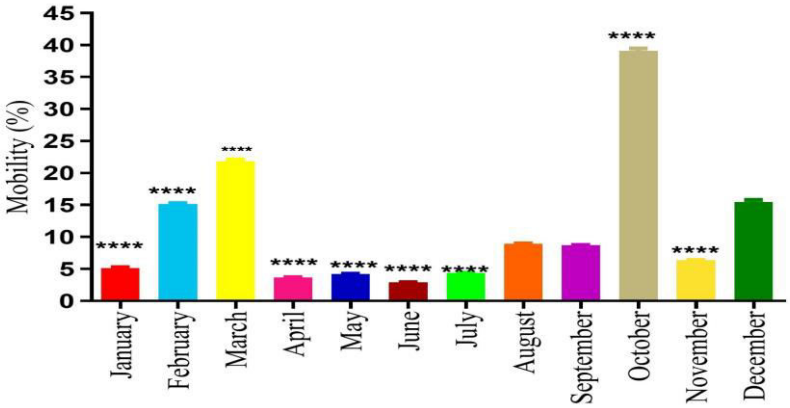

Fig. 4: Circannual behavior study of Mobility % in *Barilius bendelisis*. **** indicates P<0.0001.

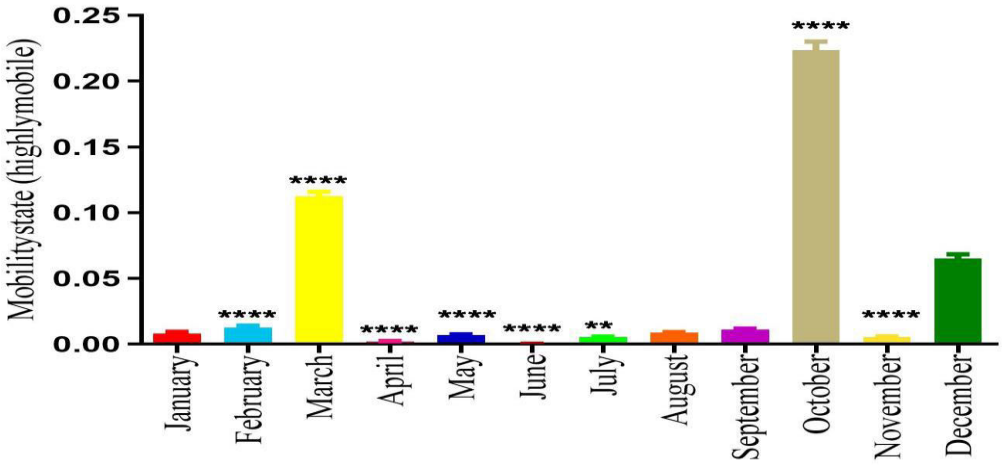

Fig. 5: Highly mobile state of *Barilius bendelisis* in circannual behavioral study. **** indicates P<0.0001.

Mobility state (*Highly mobile*):

Circannual behavior studies showed that a highly mobile state was highest in October (0.2237 ± 0.0064) and lowest in June (0.0002651 ±1.000076). One-way ANOVA analyzed all data (F (11, 76469) = 2579; P<0.0001) followed by Tukey's multiple comparison test, which showed a highly significant change among the months of the year (P<0.05) (Fig. 5).

Mobility state (*Mobile*):

During the behavioural recording of fish, the Mobile state of mobility was maximum in October (0.4618 ± 0.007) and minimum in June (0.002827 ± 0.00025). Data were examined by One-way ANOVA (F (11, 76469) = 4170; P<0.0001) followed by Bonferroni's multiple comparison test, which showed a highly noteworthy variance (P<0.05) between all months (Fig. 6).

Mobility State (*Immobile*):

The average immobile state was maximum in June (0.9969 ± 0.0003) and minimum in October (0.3145 ± 0.0067). One-way ANOVA (F (11, 76469) = 6868; P<0.0001) followed by post hoc Bonferroni's multiple comparison test exhibited a fundamental change between all months (Fig. 7).

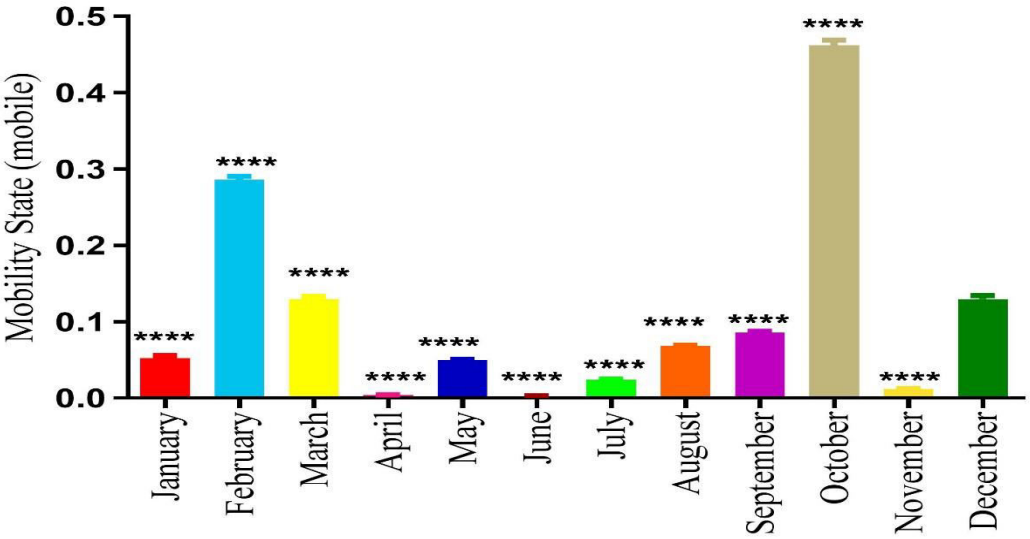

Fig. 6: Mobile state (Mobile) of *Barilius bendelisis* during circannual behaviour . **** indicates P<0.0001.

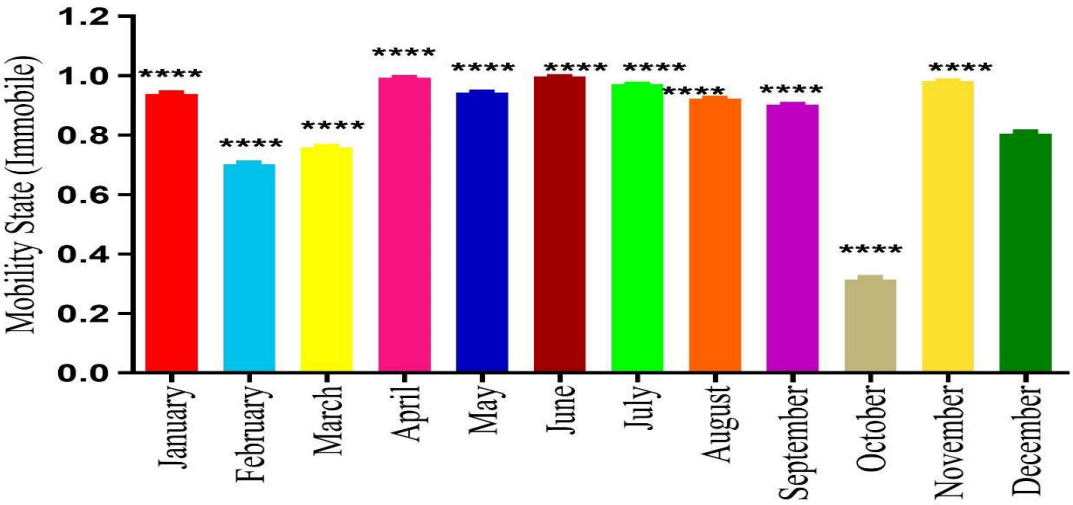

Fig.7: Immobility (Freezing) of *Barilius bendelisis* during behavioral observational tank. **** indicates P<0.0001.

Discussion

In the present investigation, the behavioral profiling of the *Barilius bendelisis* was scored monthly-wise. The fish explored the maximum Area in June 2021, while the minimum area of exploration was observed in September 2021. A highly significant difference was observed throughout the year. A similar study on *Tor putitora* after exposure to dissolved oxygen was carried out by Kalkhundiya *et al*. (2021). A similar study on the effect of lighting and ethanol on the larval form of zebrafish showed a distinct type of locomotory behavioral pattern (MacPhail *et al*., 2009). The exploratory behaviour also shows the

zebrafish's sex-specific differences (Stewart *et al*., 2010).

Fishes explore for their food niche as well as for their habitat also. The exploratory behavior is a marker of their happiness. Dopamine is a pleasure hormone of happiness among animals. A similar study on the level of dopamine in zebrafish suggested that female zebrafish had full dopamine content compared to their counterparts (Stewart *et al*., 2014). However, in the present investigation, such differentiation of the behavioral study was not studied.

Turn angle is a directional change in the swimming of fishes (Tran and Gerla, 2013). In the past, an erratic movement was characterized as stress behavior measured as the value of turn angle and angular velocity (Facciol *et al*., 2019).

Turn angle is premeditated as the alteration in the path of the epicenter point or head direction line among two successive trials (Stewart *et al*., 2014). In the present study, the turn angle (Deg) was maximum in April, while the minimum was in August 2021. The turn angle showed a discontinuous pattern throughout the year. Jyoti *et al*. (2022) worked on the effect of vitamin C on the behavioral aspect of the *Barilius bendelisis*.

Meander is the alteration in the way of the drive of an object compared to the distance it moves. So it can be used to compare the amount of turning of objects traveling at different speeds (Spink *et al*., 2001). The present circannual study of the *Barilius bendelisis* showed a significant difference throughout the year. The behavioral observation recorded a maximum meander in January compared to the lowest value in October 2021. Different concentration of donepezil (acetylcholine inhibitor) alters the value of meander in zebrafish. The value of meander after 2.5 ppm donepezil exposure showed maxima compared to natural control (Audira *et al*., 2020).

Mobility is the marker of the exploration of fishes. The mobility state was categorized into the fish's 20-80% mobile nature, whereas less than 20% is immobility and more than 80% is highly mobile. The high mobility state and immobility are considered the state of stress behavior among the fishes. In the present investigation, maximum mobility % was observed in October 2021 compared to other months. The behavioral profile of the *Barilius bendelisis* used in this experiment was graded every month. Fishes showed a high mobility state and mobility state and mobility state in October 2021 and minimum in June 2021. The locomotory behavior of rainbow trout fish was affected by the injection of the Corticotropin Releasing Factor (CRF) which increased plasma cortisol levels. Fish also exhibit increased locomotion in environments in which they are acquainted (Carpenter *et al*., 2007). In this study, immobility or freezing was studied throughout the year. Freezing showed by fish was maximum in October 2021 and minimum in June 2021. According to Egan *et al*. (2009), zebrafish showed less exploration, irregular movements, and freezing behavior in response to acute alarm pheromone and acute caffeine, generating anxiogenic solid effects. A similar study was reported on *Poecilia reticulate* (Inbred Guppies), which exhibited freezing behavior in response to predator stimuli (Bleakley *et al*., 2006).

Conclusion

The present circannual behavioral finding indicated the monthly profiling of the hill stream fish *Barilius bendelisis*. This study provided the baseline data for the future rational management and conservation of these fishes and was helpful in their applied aspect of neuropharmacology.

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