



## The Behavioral Profiling of Freshwater Hill stream Fish *Barilius bendelisis* (Hamilton) After the Exposure of Vitamin- C

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**Abstract:** Behavioural profiling of the fish is an output of all the internal and external interactions. *Barilius bendelisis* is a Hill trout confined in the hillstreams. In the present investigation, different doses of vitamin c were given to the *Barilius bendelisis*. The exposed fishes were studied concerning their growth, chronic stress hormone cortisol by using enzyme-linked immunosorbent assay as well as their behavioural profiling with the help of Software Ethovision XT 13 (Noldus, infotech, Wageningen, the Netherlands). In the present study, vitamin c plays a significant part in the growth performance and the behavioural scoring of swimming speed, turn angle, travelled distance and area of exploration. The behavioural profiling and growth performance exhibited a significant difference at  $P \leq 0.05$ .

**Key Words:** *Barilius bendelisis*, Growth, Ethovision XT 13, Behavioural profiling, Cortisol

### Introduction

*Barilius bendelisis* also known as *Opsarius bendelisis* (Kottelat, 2013). *Barilius bendelisis* belongs to Cyprinidae and this family includes cultivable fish species. (Badola and Singh, 1980). It is generally recognized as “Indian Hill Trout”. It is a common freshwater fish of ‘South East Asia’(Sahoo et al.2009). It is locally known by different names such as Chadu, Buddi, Jhojha etc. *Barilius* is a surface-feeder omnivorous ornamental fish and has great food value. According to ‘The International Union for Conservation of Nature & Natural resources’(Vishwanath, 2010), the status of *Barilius bendelisis* was categorized as of ‘least concern (LC)’, But in upcoming times, it is expected that over suction and demolition of habitat may pose a foremost danger to its fishery (Sukham et al., 2013). Ascorbic acid is included in the group of water-soluble vitamins. Which is synthesized by most animals and plants (Debora M. Fracalossi, 2001). Due to the shortage of L-

gluconolactone, most teleost fishes can’t synthesize ascorbic acid and need ascorbic acid as a dietary supplement (Ali et al. 2004, Fracalossi et al. 2001). Ascorbic acid is a significant vitamin for the development and reproductive processes of many fish species and it is a fundamental micronutrient for fishes because it helps to decrease stress and acts as a metabolic antioxidant, protecting cell membrane and intracellular components and it also acts as a cofactor in the combination of collagen which is involved in the creation of blood vessels, tissues, bone matrix(Ibrahem et al., 2010). Deficiency of vitamin C in fishes can cause reduced growth, structural deformities such as scoliosis & lordosis, a weak immune system, the fragility of capillary increase, decrease in reproductive performance (Fracalossi et al., 2001). Based on metabolic functioning and form of vitamin C, a requirement of vitamin C differs such as when ascorbic acid used as a dietary source, in which 3000 mg/Kg is sufficient in the food of channel catfish for best growth and in the constant form



of vitamin C, 11- 45 mg/kg food was enough for best growing (Lim et al., 2000). The study on the consequence of diverse dosages of vitamin C (Durak et al., 2013) on the growth of fishes has been supported by many researchers (Ibrahim et al. 2010, Adel & Khara, 2016, Ali et al. 2004, Lim et al. 2000, Ion et al., 2012, Ibiyo et al. 2006 and Kumari & Sahoo, 2005).

Nearby is no investigation on the consequence of vitamin C on *Barilius bendelisis* concerning their behavioural profiling. So, the present investigation aimed at the outcome of diverse dosages of vitamin C on the behaviour profiling of *Barilius bendelisis*.

### Materials and Methods

Adults of *Barilius bendelisis* were collected from Suwalekh stream Pithoragarh Uttarakhand (29.66°N, 80.22° E), and transported to the laboratory and acclimatized to normal ecological circumstances in a laboratory. Fishes were fed two times a daytime with artificial food. After acclimatization 15 adults of *Barilius bendelisis* (initial body weight- 3.26gm – 7.49gm) were selected and equally divided into three tanks (5 fishes in each tank). The experiment was conducted in an aquarium (32×45×30cm) for 78 days. Each group was given different dosages of vitamin C.

- 1). Control i.e., Natural condition.
- 2). Low dose of Vitamin C with food (LdF) i.e. food & vitamin C in the ratio of 9:1 ( 9gm food+1gm vitamin C).
- 3). High dose of vitamin C with food (HdF) i.e. food & vitamin C in the ratio of 8:2 (8gm food+2gm vitamin C).

Each tank was filled with 70% of water volume. All tanks were continuously aerated by using air pumps and air stones. Removal of excretory material and renewal of 70% water has been done every four days. Fishes were nourished two times in the daytime i.e. in the morning and after 6 hours in the evening. Fishes were given artificial

food (crude protein 32%, crude fat 4%, crude fiber 5%, crude ash 10%, Nitrogen 31%, moisture 9%). Experimental food pellets were prepared by crumbling artificial food pellets and adding different dosages of vitamin C (Limcee vitamin C chewable tablets 500mg) & water then mixed well and made pellets from this mixture and were dried at room temperature for 24 hours. During the experiment, food was given 2.5% of the total body weight of the tank for 78 days. The weight of each adult fish was recorded in an electronic balance with a precision of 0.001 gm (Roy Electronics India model no. CBCAB300). Body mass was measured at the beginning of the experiment and repeated every tenth day of an experiment as well as at the termination of the experiment. After 78 days fishes were anaesthetized through clove oil. The cortisol was extracted as per the protocol (Cachat et al., 2010). The extracted cortisol level of fishes was measured by an Enzyme-linked immune sorbent assay kit (Cayman chemical, Cortisol ELISA Kit).

### Behavioural study

For behaviour study, the video of individual fish was recorded in an open square tank (50×30×30 cm) with the help of a digital Basler camera with pylon four-camera and behaviour was computed through Ethovision XT 13 Software' (Noldus, infotech, Wageningen, the Netherlands) for 300 sec at the rate of 25 frames/sec. A few behavioural parameters were calculated such as explored area, Distance travelled, Body elongation, Velocity and turn angle of fish were studied.

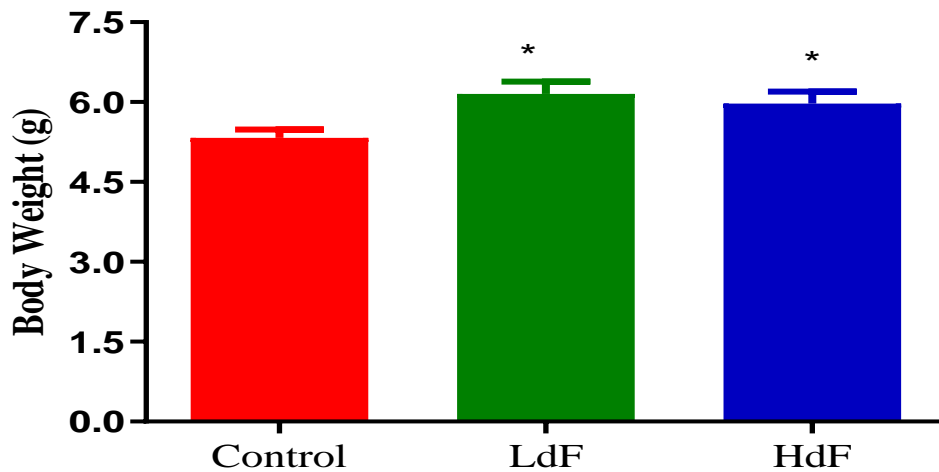
**Statistical analysis:** The statistical examination was done by one way ANOVA followed by post hoc test such as 'Newman-Keuls Multiple comparison test and Dunnett's multiple comparison test' using GraphPad Prism.

### Results



In this experiment, the average body weight of fish *Barilius bendelisis* was highest in the LdF group ( $6.15 \pm 0.23\text{g}$ ) and HdF group ( $5.98 \pm 0.22\text{g}$ ) than in the control group ( $5.33 \pm 0.15\text{g}$ ). Analysis of body weight was done by using 'One Way

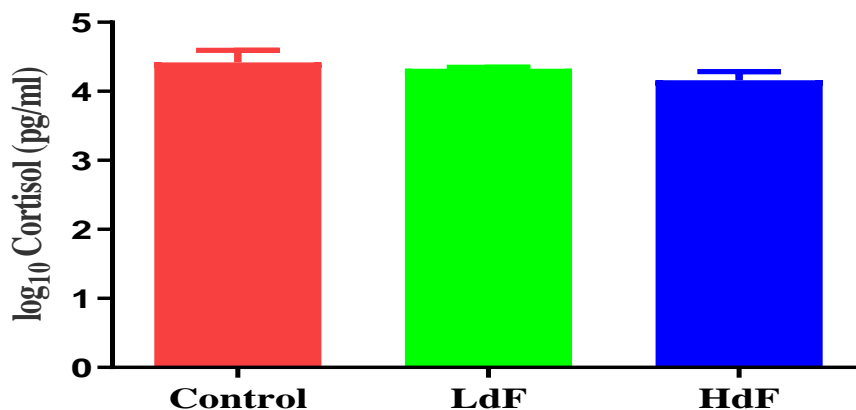
ANOVA ( $F(2, 21) = 4.438$  at  $P \leq 0.0247$ ) analysis followed by Newman-Keul's Multiple Comparison test' which showed a significant difference between bodyweight of Control and other groups (LdF-HdF) at ( $P < 0.05$ ), (Fig.1).



**Fig.1.** The body weight of *Barilius bendelisis* after the exposure to vitamin c (Mean±SE)

Cortisol concentration of *Barilius bendelisis* in the control group was ( $32825 \pm 11999$  pg/ml), in the LdF group ( $21309 \pm 1053$  pg/ml) and HdF group was ( $16508 \pm 5342$  pg/ml), The measured

data was log-transformed and analyzed by using One Way ANOVA ( $F(2, 9) = 1.083$ ,  $P \leq 0.38$ ) followed by post hoc Dunnett's multiple comparisons test at of all groups (Fig 2)



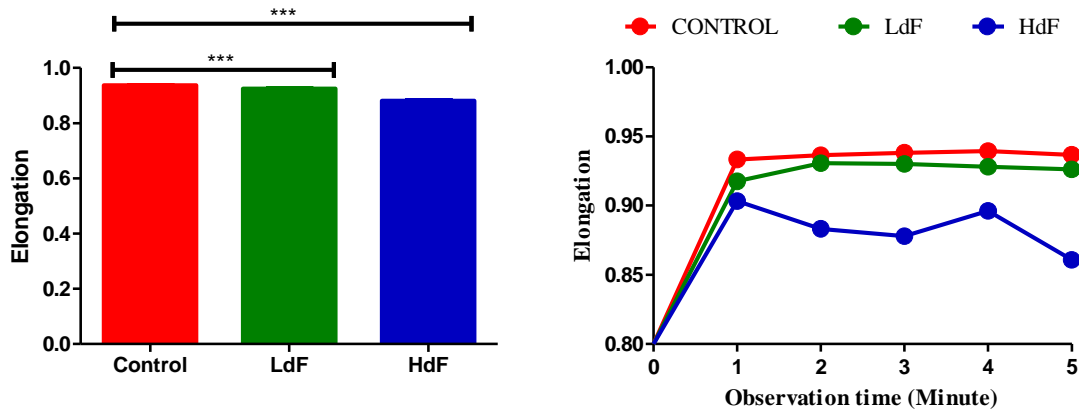
**Fig.2.** The average Cortisol Concentration (pg/ml) of the Vitamin-C exposed fishes

During the behaviour study, the body elongation of *Barilius bendelisis* was  $0.9370 \pm 0.0005$ ,  $0.9251 \pm 0.0010$  and  $0.8814 \pm 0.0014$  was recorded



in control, LdF and HdF groups respectively. Examination of these statistics was completed by using ‘One Way ANOVA followed by Dunnett’s

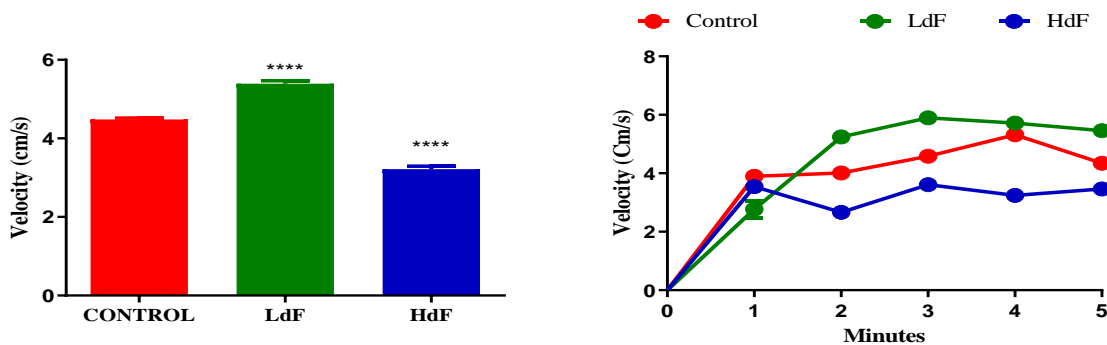
Multiple Comparison Test’ which showed a noteworthy difference ( $P < 0.0001$ ) between body elongation of all groups (Fig.3).



**Fig.3. Body Elongation of *Barilius bendelisis* after Vit. C treatment.**

Average Velocity of control group was  $4.48 \pm 0.04$  cm/sec, LdF group  $5.39 \pm 0.08$  cm/sec and HdF group  $3.22 \pm 0.07$  cm/sec. The data of velocity was analyzed by using the One Way

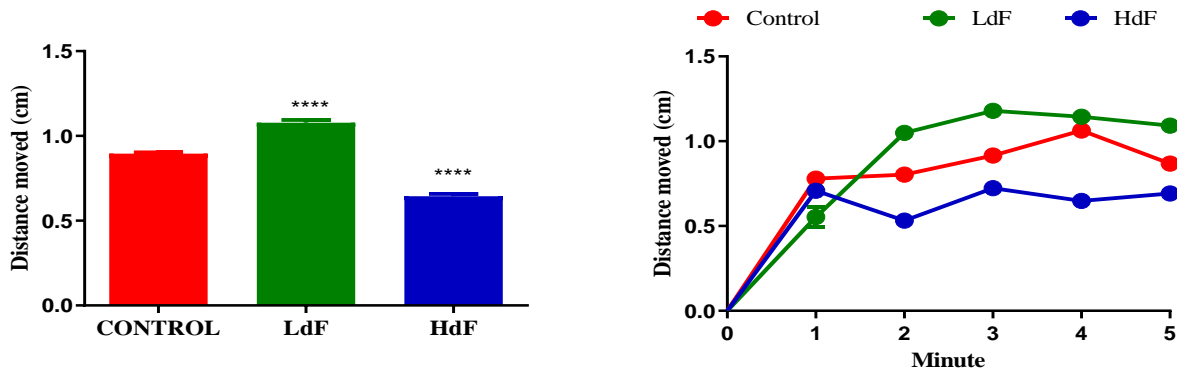
ANOVA ( $F(2, 4171) = 283.2$  at  $P < 0.0001$ ) ‘followed by post hoc Dunnett’s multiple comparison test which showed a significantly different from each other  $P < 0.0001$  (fig.4).



**Fig.4. The average velocity of *Barilius bendelisis* during behaviour observational duration**

The average travelled distance in the control ( $0.89 \pm 0.01$  cm), LdF group ( $1.08 \pm 0.02$  cm) and HdF group ( $0.64 \pm 0.02$  cm). The total distance travelled by fish during the behavioural observation was subjected to the one way

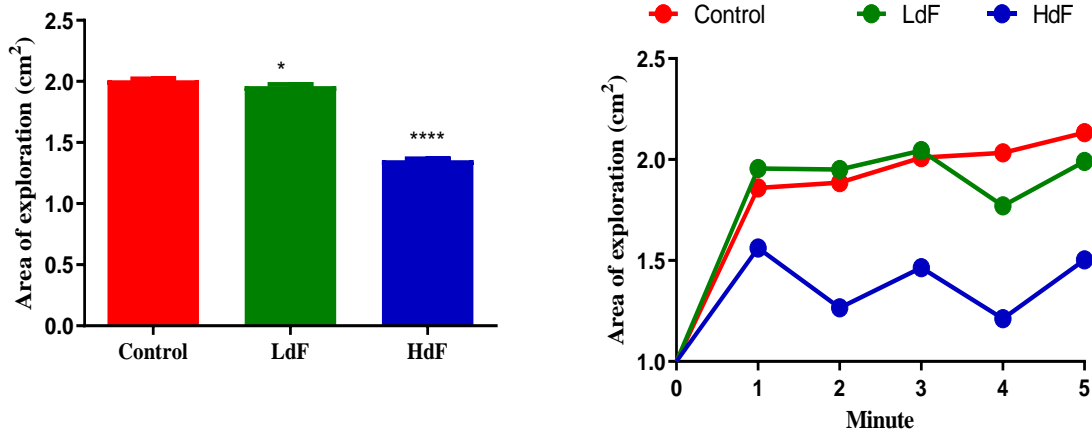
ANOVA analysis. one way ANOVA ( $F(2, 4171) = 283.2$  at  $P < 0.0001$ ) analysis showed a highly significant difference followed by post hoc Dunnett’s multiple comparison test (Fig.5).



**Fig.5.**The distance travelled by *Barilius bendelisis* during the observational period (Mean±SE)

The area explored by fish was minimum in the HdF group ( $1.36 \pm 0.01 \text{ cm}^2$ ), in the LdF group ( $1.96 \pm 0.01 \text{ cm}^2$ ) and maximum in the control group ( $2.01 \pm 0.01 \text{ cm}^2$ ). One way ANOVA F (2,

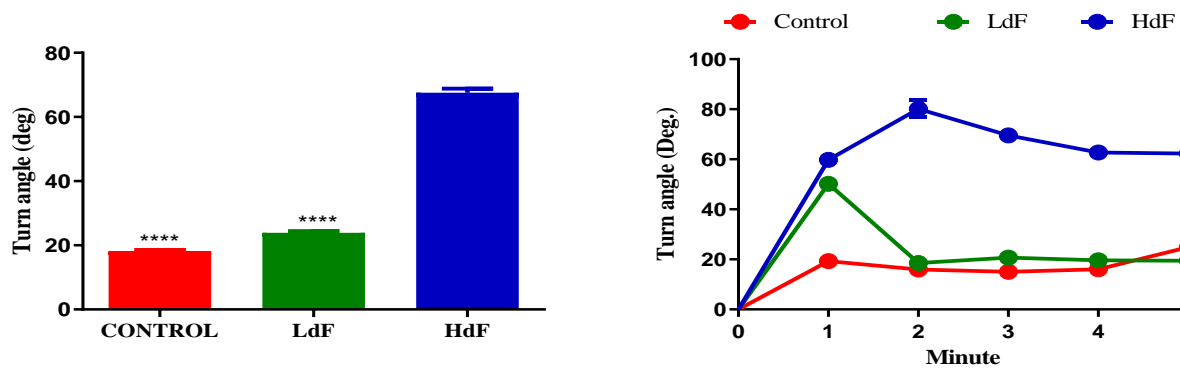
4171) = 858.3 at  $P < 0.0001$ ) analysis followed by post hoc Dunnett's multiple comparison test showed a significant difference between the travelled distance of all groups at  $P \leq 0.05$  during the behavioural observation Fig 6.



**Fig.6.** Average explored area of the arena by *Barilius bendelisis* during behaviour observation

The turn angle of fish in the control group was  $18.23 \pm 0.33 \text{ deg}$ , in the LdF group was  $23.89 \pm 0.53 \text{ deg}$  and in the HdF group was  $67.57 \pm 1.25 \text{ deg}$ . The data of turn angle was

subjected to the one way ANOVA(F (2, 4171) = 1064 at  $P < 0.0001$ ) analysis followed by Dunnett's multiple comparison test showed a noteworthy difference between turn angles of all groups at  $P \leq 0.05$  (Fig.7).



**Fig.7.**The average Turn angle of *Barilius bendelisis* during behavioural trial

### Discussion

Vitamin C is essential for fish in many ways such as growth of fish, reproduction and works as an antioxidant. It also acts as a cofactor for the synthesis of collagen (Suwanmanee et al. 2012). According to Ai et al. (2004) and Fracalossi et al. (2001), most teleost fishes have a shortage of enzyme ‘L-Gulonolactone oxidase’ which is involved in the formation of Ascorbic acid. L-Gulonolactone oxidase catalyzes the alteration of L-Gulonolactone into Ascorbic acid. Due to a shortage of L-Gulonolactone, oxidase teleost fishes can’t synthesize Ascorbic acid so they need ascorbic acid in their dietary supplements. The present study shows that the growth of adult *Barilius bendelisis* is enhanced by the Vitamin C supplements in the ratio of 9:1 (food: Vitamin C, i.e. LdF). Vitamin C in the ratio of 8:1 (food: vitamin C, i.e. HdF) also stimulates the growth of adult *Barilius bendelisis* (Fig 1). There are many other studies related to the outcomes of vitamin C on the development of fish, similar to the current experiment. The experimental studies of Ahmed et al. (2018) and Ibrahim et al. (2020) on *Oreochromis niloticus* showed high dose (i.e. 400mg/kg) of vitamin C is best for the growing of fish. Similar studies on *Labeo rohita* also exhibited that vitamin C affects the growth of *Labeo rohita* (Tewary & Patra,

2008, Ahmed et al., 2018). The current study exhibited that the cortisol level is maximum in the control group and lowermost in the HdF group. Vitamin C acts as an anti-stressor. Ortuno et al. (2003) have studied that vitamin C helped in reducing induced stress in fish *Sparus aurata*. Vitamin C provides resistance against different kinds of stress to *Labeo rohita* (Laltlanmawia et al. 2019) as well as from the tilapia like viruses in the Tilapia (Vineetha et al. 2021). Bo Liu et al. (2016) reported that vitamin C decreases stress caused by high pH in fish *Megalobrama amblycephala*. The present study indicates that a maximum dosage of vitamin C can reduce the levels of cortisol in *Barilius bendelisis* the lowest level of cortisol indicated in the HdF group irrespective of a significant difference (Fig 2). During the behavior study movements of fish were recorded with the help of Ethovision XT 13. Different movements such as area explored, distance travelled, velocity, body elongation and turn angle have been recorded to expressing of the behavioural profiling of fish. As per the research of Cachat et al. (2010), in a stressful environmental condition, zebrafish reduces area exploration, freeze & dive, as it adapts to the environment, which increases area exploration & decreases freezing.



The stress created due to toxicant exposure can affect the behavioural responses of fish. These behavioural responses are change in locomotor activity such as increased percentage of movement, frequency, speed of swimming (velocity) & distance from the center (Kane et al. 2004). According to Stewart et al. (2015), behaviour, treatment of nicotine (anxiolytic compound) to zebrafish causes an increment in swimming, increased distance travelled by fish and turn angle decreased. In this study velocity & distance travelled are uppermost in the LdF group and lowermost in the HdF group (Fig 4,5). The explored area is maximum in the control group and lowermost in HdF group. The turn angle is maximum in HdF group and bottommost in the control group. Kalkhundiya et al. (2021) worked on exposure of the dissolved oxygen and its impact on the behavioural profiling of the Coldwater fish Golden Mahseer. They concluded that the high dissolved oxygen content affects the area of exploration in the *Tor putitora*. This study concludes the role of vitamin c on the alteration of behavioural profiling of *Barilius bendelisis* concerning their swimming speed, Turn angle and area of exploration and the travelled distance.

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