

# Behavioral Assessment of Koi carp (*Cyprinus carpio*) Exposed to Acidic and Alkaline pH

**Proteek Dasgupta\***

Reproductive Physiology Lab  
Department of Zoology,  
Bangalore University, Jnana Bharathi Campus,  
Bengaluru – 560056, Karnataka, India

**Duraiswamy Usha Anandhi**

Reproductive Physiology Lab  
Department of Zoology,  
Bangalore University, Jnana Bharathi Campus,  
Bengaluru – 560056, Karnataka, India

\*Corresponding Author - **Proteek Dasgupta\***,

**Abstract:-** This study presents qualitative and behavioral evaluations in Koi carp (*Cyprinus carpio*) exposed to acidic and alkaline pH. Renewal system maintained for 96 hours was observed intermittently every 12 hours from pH 4 to 10. Qualitative indicators evaluated were (1) Repeated movement (2) Abrupt swimming (3) Body fatigue (4) Loss of physical activity (5) Loss of appetite (6) Change in body colour (7) Burnt marks (8) Decolouration of muscles (9) Mucous discharge (10) Glossy gills (11) Shedding of scale and (12) Disintegrated body parts. There was no mortality throughout the study. Majority of behavioral aversions and irregularities were observed at acidic (4 and 5) and alkaline (9 and 10) levels. The study suggests that this species is best maintained between pH of 6 to 8 in a domesticated subsystem.

**Keywords:-** Acid, Alkaline, Carp, Exposure, Ornamental Fish.

## I. INTRODUCTION

Water parameters always influence various biological processes in aquatic ecosystem. As an important water parameter, there exists a profound correlation between sensitivity of fish to pH. Exposure to pH outside acceptable range can adversely affect metabolism [1]. Acidification of water bodies have been reported to impact fish health and physiology [2]. This is even extended to DNA damage and alterations in the blood biochemistry [3]. Abrupt pH fluctuations are more stressful than consistent exposure to individual values [4]. However, in every possible scenario, evaluating changes in behavior can reveal primary signs of discomfort and aversiveness.

Koi carp (*Cyprinus carpio*) is an ornamental fish with global preference due to aesthetics and colouration varieties. The presence of barbels is a distinct feature of this species. It is generally found in lakes, ponds and water streams and majorly used for decorative purposes. It is an exotic preference for aquarist and is commercially prevalent in retail outlets for domestication and aquarium subsystems [5, 6].

In a subsystem like water tank, fish is strictly confined to a specified dimension to navigate and swim. Therefore, pH must be strictly regulated and monitored in the water tanks to avoid health issues leading to mortality. Even more, it becomes crucial to do so, because fecal matter might influence pH. Therefore, the present study aims to understand first signs of qualitative and behavioral indicators in Koi carp exposed to wide range of acidic and alkaline pH.

## II. MATERIALS AND METHODS

### A. Fish Acclimation

Fish were collected from Ornamental Fish Research Centre, Bengaluru. Juveniles ( $4.61 \pm 0.34$  g) were acclimated in lab for 14 days maintained under natural daylight conditions ( $\approx 12$  L:12 D). Glass tanks (50 L capacity) were installed with aerator and thermostat. Fish were fed twice a day with commercial fish feed (Taiyo, India). The water parameters were checked for oxygen (7.2 mL/L), temperature ( $25 \pm 1^\circ\text{C}$ ) and pH ( $6.99 \pm 0.1$ ). Tanks were covered with mosquito nets and cleaned on alternate days to prevent fecal toxicity. Water standards were maintained in accordance with APHA [7].

### B. Experimental Setup

Exposures were maintained in duplicate tanks with 5 fish in each ( $n = 10$  per exposure) for a duration of 96- hours (= 4 days). The acclimation tanks were marked as control group. The acidic and alkaline compositions were made up of dilutions of hydrochloric acid and sodium hydroxide. In order to avoid interference of fecal toxicity, tanks were cleaned with suction pumps and pH was adjusted again with dilutions of the respective acid and alkali. The tank pH levels were regularly monitored with a digital meter (Systronics MK-VI) with a discrepancy of  $0 \pm 0.5$  pH units. Feeding regime and water parameters were similar to the acclimation phase. Fish were fed to avoid starvation effects during experimental evaluation.

### C. Behavioral Indicators

A total of 12 behavioral indicators were evaluated every 12 hours. Therefore, eight evaluations were made during the 96- hour period. Indicators evaluated were (1) Repeated movement (2) Abrupt swimming (3) Body fatigue (4) Loss of physical activity (5) Loss of appetite (6) Change in body colour (7) Burnt marks (8) Decolouration of muscles (9) Mucous discharge (10) Glossy gills (11) Shedding of scale and (12) Disintegrated body parts.

### III. STATISTICAL ANALYSIS

The statistical tests were computed in GraphPad Prism (Version 5). Correlational analysis was used to tabulate the indicators for the acidic and alkaline (Table 1 & 2) exposures. Significant differences were calculated by One way ANOVA.

### IV. RESULTS

There was no fish mortality during the experimental period. Majority of the indicators were dominant at pH of 4, 5, 9 and 10. These levels were marked by body fatigue, loss of appetite, mucous discharge, scale shedding, glossy gills, burnt body marks and disintegrated body parts. Dorsal and caudal fin were blistered and substantially disintegrated at 4, 9 and 10. At low pH levels, gill appeared extremely glossy due to excessive mucous covering the lamellae.

### V. DISCUSSION

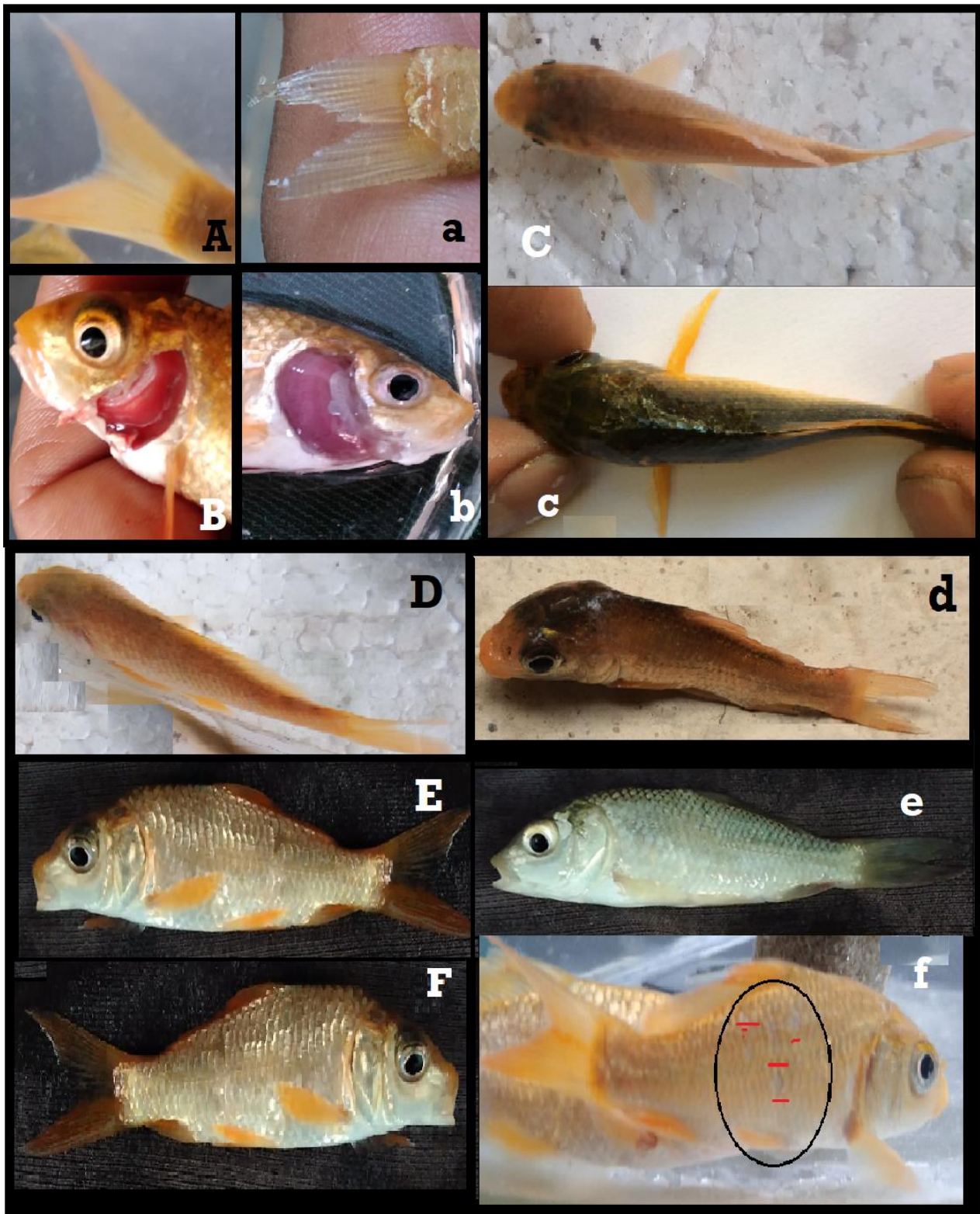
The present study qualitatively assessed the behavioral and morphological damages in Koi carp exposed to pH 4 to 10. Abrupt swimming was observed at most of the pH levels, and exceedingly at 4, 9 and 10. Sudden strokes and jolting were also observed, indicating an uncomfortable environment. This also included very few repeated movements from diagonal ends which clearly exhibited anxiety and avoidance to that pH level. This kind of behaviour has also been observed in Snapper (*Pagrus auratus*), Yellowfin bream (*Acanthopagrus australis*), Australian bass (*Macquaria novemaculeata*), School prawn (*Metapenaeus macleayi*) and Silver catfish (*Rhamdia quelen*) [8, 9].

Mucous discharge from the lateral sides of the jaw was also prevalent at pH 4, 5, 9 and 10. Previous literature reveals that extreme acidic and alkaline levels interfere with gill histochemistry. This ultimately results in hypertrophy and the gill surface becomes glossy due to excessive secretion of mucous. Consequently, coagulation of the gill mucous can lead to asphyxiation and mortality [11]. Such phenomenal observations reported in Brook charr (*Salvelinus fontinalis*) [10] resonate in the present study (Fig 1B and b).

Decolouration of body and muscle was also observed at both the near-end acidic and alkaline levels. Body colour changed from bright vermilion into whitish grey (Fig 1E and e). Decolouration of the muscle and shedding of scales was prevalent at pH of 4 and 10. Majority of shedding took place within the first 24- hours and the shed scales were corroded (Fig 1F and f).

Body fatigue was observed at all the levels except at control, though it was almost negligible at 6 and 8. The remaining levels proved to be difficult for the adaptation. Consequently, fish showed no physical activity at 4, 9 and 10. Fish displayed sedimentary behavior occupying one corner of the tank for a long duration. Perhaps loss of appetite aggravated such lethargy as observed in Nile tilapia (*Oreochromis niloticus*) [12].

Fish were depressed at extreme acidic and alkaline levels. Fusiform body became compressed from the lateral sides behind the pectoral fins (Fig 1D and d). Dorsally, burnt black marks (Fig 1C and c) were seen running from anterior to posterior end, conspicuous at pH 4 and 10. These levels also showed disintegrated dorsal and caudal fins (Fig 1A and a). Instances of burnt dorsal surface and damaged fins have already been reported in Nile tilapia (*Oreochromis niloticus*) [12].



**Fig 1** – Visual Representations of Koi carp Exposed to Acidic and Alkaline pH. **A** – Normal Caudal Fin **a** – Disintegrated Caudal Fin; **B** – Normal Gill **b** – Glossy Gills; **C** – Normal Dorsal Surface **c** – Burnt Dorsal Surface; **D** – Lateral Region Under Normal Conditions **d** – Deformed Lateral Region; **E** – Normal Body Colour **e** – Decolouration; **F** – Scale Under Normal Conditions **f** – Lesions without Scale (Indicted by -)

**Table 1** – Tabulation of the Behavioral Checkpoints in Koi carp (*n* = 10 per Exposure) Exposed to pH from 4 to 10 with Neutral pH 7 (Control). Behavioral Intensity is Represented by Dots (**Blank - Absent; • - Mild; •• - Moderate; ••• - Strong**) for the Respective pH Indices.

CHECKPOINT	pH range						
	4	5	6	7	8	9	10
Repeated movement	•	•			••	•	
Abrupt swimming	••				••	••	••
Body fatigue	••	••	•		•	••	••
Loss of physical activity		•			•		
Loss of appetite	•••	•••	•		•	••	••
Change in body colour	•••	••			•		•••
Burnt marks	•••	••	•			•••	•••
Decolouration of muscles	••	•	•				••
Mucous discharge	•••	••	•			•••	•••
Glossy gills	••	•					•••
Shedding of scale	•••	•	•		••	••	•••
Disintegrated body parts	•••	••				•••	•••

**VI. CONCLUSION**

The results indicate that Koi carps can be well maintained between pH of 6 to 8. Further, regular cleaning of the tanks is mandatory to prevent fecal toxicity that might influence the pH levels.

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