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Effect on Growth Performance, Hematology and Condition Factor of the Common Carp (*Cyprinus carpio* L., 1758) Using Challenged System

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ABSTRACT

The pathogenicity of *Pseudomonas* sp. on the length-weight relationship and hematology of the cultured fish *Cyprinus carpio* were addressed in the current study. Fish samples were intraperitoneally injected with an experimental bacterial solution, containing 2.0×10^6 CFU/ml at a dosage of 0.2ml. Significant behavioral changes were detected in the infected fishes, as well as a reduction in length and a loss in body weight. The results reveal that the controlled fish have a significant increase in length and weight, but the infected fish exhibit no significant growth at subsequent time intervals of the experiment. The CFs from infected populations were less than 1.0, suggesting that the fish population has not achieved its expected growth. The hematological data, such as hemoglobin (Hb) concentration, total erythrocyte count (RBC), total leucocyte count (WBC), hematocrit, packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) were analyzed. The findings showed that while the number of WBC rises, the hematological parameters (Hb, RBC, and PCV) exhibited a substantial drop in the count with regard to a time interval of infection. Thus, the study showed that *Pseudomonas* affected the infected host fish's development, hematology and behavioral changes in the cultured host fish.

INTRODUCTION

The common carp, *Cyprinus carpio*, is most widely cultured freshwater fish species in the world **(Sterniša *et al.* 2020)**. It may be found in ponds, lakes and slow-moving rivers **(Yaqoob, 2021)**. It enjoys warm water although it can also endure cold water, pollution, and low dissolved oxygen levels. Common carp has been successfully introduced into most freshwaters across the world due to its quick development and ability to adapt to difficult environmental conditions **(Seegers *et al.*, 2003; Rypel, 2014)**. Since it is available all year, it is an important source of animal protein for humans all over the world. The most popular farmed freshwater fish in Kashmir is common carp, which is second only to rainbow trout. One of the most important aspects of successful aquaculture is effective disease management **(Cooper & Shlaes, 2011)**. To adjust a high number of fish in a confined space creates an ideal setting for the development and spread of infectious illnesses. Fish are stressed and more prone to infections in a crowded artificial habitat. The bacterial infections are the most prominent microbiological agents impacting cultured fish, and *Aeromonas* sp. and *Bacillus* sp. were most prevalent in fish microbiota **(Wani *et al.*, 2022)**. Fish haematological findings may be affected by biological and environmental factors, viz. age, weight, sex, nutrition, bacteria, parasites and water quality parameters including temperature, salinity, oxygen, availability and pH **(Ahmad *et al.*, 2020)**. Understanding the length-weight relationship (LWR) is especially important in the bio-ecological study of fish since it provides information on population growth trends, as well as general health because the length-weight relationship in an aquatic habitat varies based on environmental circumstances. The LWR in fishes can be used to calculate the fish's mean weight from its length **(Koutrakis & Tsikliras, 2003)**, study fish population growth and convert growth-in-length equations to growth-in-weight for the prediction of weight at age **(Costa *et al.*, 2018)**, as well as predicting gonad development, maturity and other phenological characteristics of that fish individual **(Servili *et al.*, 2020)**. Fish weight may be estimated from length or vice versa using length-weight relationships **(Xiong *et al.* 2015; Zhu *et al*. 2015)**. Hematological testing is one of the most frequent ways for determining the physiological condition and health of fish **(Ivanc *et al.* 2005; Clauss *et al.* 2008; Grant, 2015; Docan *et al.* 2018; Fazio, 2019)**.

In light of the foregoing hypotheses, the current study was conducted in order to derive critical conclusions regarding the LWRs parameter of the most numerous fish species, common carp, residing commonly in local farm in Kashmir Himalaya, India for future management and conservation measures.

MATERIALS AND METHODS

2.1 Fish collection

The common carp, *C. carpio* (14±3 cm) individuals with weight between 21.5-29.0g were obtained from fish farm Rangril SKUAST, India.

2.2. Experimental fish

Fish samples were acclimatized in the microbiology laboratory at the CORD, University of Kashmir, India, unmoved and kept in static recirculating water (27°C) overnight; they received a gentle NaCl (2%) treatment for two minutes the next morning. Each fish tank (80 x 58 x 40 cm, 150 l) was cleaned with KMnO4 solution (4 mg^{-1}). Before the fish were distributed, a total of 90 fish were randomly assigned to one control group and one experimental group, each with three replicates (15 fish × 6 tanks = 90 fish). Fish were stocked in 24 tanks (200 l capacity) for a month. This experiment's acclimation approach followed that of **Das *et al.* (2021)**. Rearing experiment tanks were positioned inside an experimental space, which included lighting to supplement the natural light. Fish were given a practical diet once a day (35 % crude protein). To ensure the quality of the water, half the water in each tank was replenished each day with filtered water, and any dead fish were removed. To keep the concentrations of dissolved oxygen at saturation, additional aeration was also applied.

2.3. Artificial infection study

The fish pathogenic strain *Pseudomonas* sp. identified by different biochemical tests have been cultured on a nutrient agar in Advanced Research Lab, Department of Zoology, University of Kashmir. The bacteria were then cultured on selective medium (Hi-Media). Bacterial suspension of the test organisms was prepared in broth 1×10^7 CFU **(Mukherjee & Ghosh, 2016)**.

After that, 1×10^7 CFU bacterial suspension of the test organisms was produced in broth, and 0.2 ml of 2.0×10^6 CFU/ml of the isolated bacterial solution were injected intraperitoneally in each experimental fish using the 1.0 ml syringe. The control fish were given 0.2 ml of bacterium-free 0.4 percent (TSA) nutritional broth. Fish have been maintained in aquaria with fresh tap water. The laboratory methods and tests were carried out in accordance with the (CPCSEA) Animal Experimentation and Ethics in India, 2018 recommendations.

2.4. Behavior study

The infected and uninfected fish were subjected to behavioral study. The effects of *Pseudomonas* sp. on host fish were explored in this study by looking at several behavioral features such as swimming and eating behavior.

2.5 Changes in Growth

The linear regression analysis was used to determine length-weight connections (LWRs). The length and weight of fish after 5, 15, 25 and 35 days were tabulated. The relation coefficient (r2) was used to calculate the degree of relationship between L and W. The value of exponent b in the length-weight relationship offers information on fish growth. When $b = 3$, the weight gain is isometric. If $b > 3$, the rise in weight is positive allometric; however, if $b < 3,4$, the increase in weight is negative allometric.

2.6 Collection of blood sample

Sampling was performed 10hrs after the last feeding by puncturing the caudal puncture. Before collecting blood samples, no anesthetic was applied to fish as it may affect blood parameters and hemolyse tissues. EDTA and sodium heparin (5000 IU in 1 ml injection) were used as anticoagulants, the former being used for the hematological examination.

2.7 Condition factor

The condition factor (k) was calculated from the relationship, K = 100W/L , where W = weight (g); L = total length (cm), and b = regression coefficient. The regression analysis was carried out to analyze the data with the help of SPSS software version 22.

RESULTS

3.1. Bacteriological assay

All recovered isolates were Gram-negative, motile and organised in double or short chains, according to the bacteriological analysis. The colonies responded favorably to gelatin hydrolysis, citrate utilisation, mannitol fermentation, catalase, oxidase, nitrate reduction and nitrate reduction but negatively to H2S generation, urease, Voges Proskauer, indole and methyl red. The visual and biochemical traits allowed for the identification of all isolates as *Pseudomonas*. On a nutrient agar (NA) at 37°C for 24 hours, the usual isolates of *Pseudomonas* developed big irregular colonies with a fruity odor (Fig. 1).

Fig. 1: Isolated *Pseudomonas* on nutrient agar

3.2. Behavior and survival rate of the examined fish

Fish species (both control and infected) were daily examined till the end of the experiment. A gradual ceased movement, abnormal swimming, gasping at the surface of water, lethargic and low feeding efficiency were observed in infected fish, compared to the control groups. Furthermore, at the end of experiment, most infected fish were seen floating dorsal side down at the water surface. The survival percentage was measured at four intervals (5 days , 15 days, 25 days and 35 days) of the pathogenic bacterium strain *Pseudomonas* sp., and the survival percentage was recorded to be 82.22%, 57.77%, 44.44% and 17.77% respectively. Similarly, the survival percentage was measured at the same four intervals (5 days , 15 days, 25 days and 35 days) in controlled fish, and the survival percentage were 100%, 95.55%, 88.66% and 77.77%, respectively (Fig. 2). Fishes are always surrounded by a wide range of bacteria.

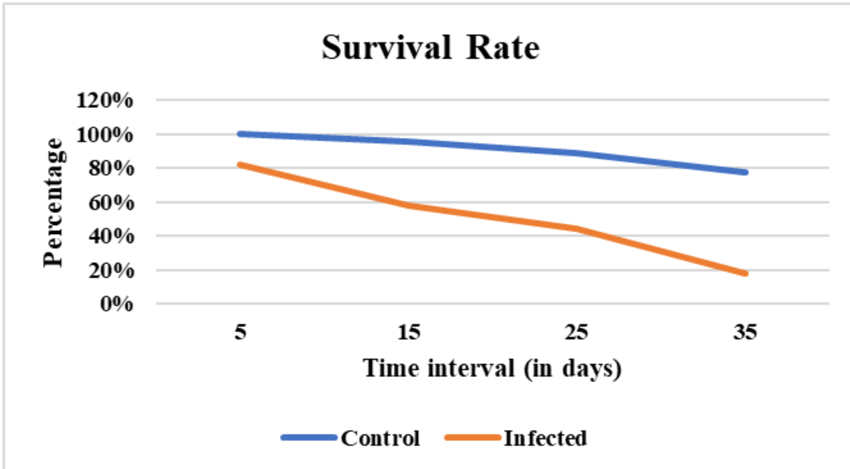


Fig. 2. The survival rate of common carp after infection with bacterial isolates at different time intervals

3.3. Challenge trial

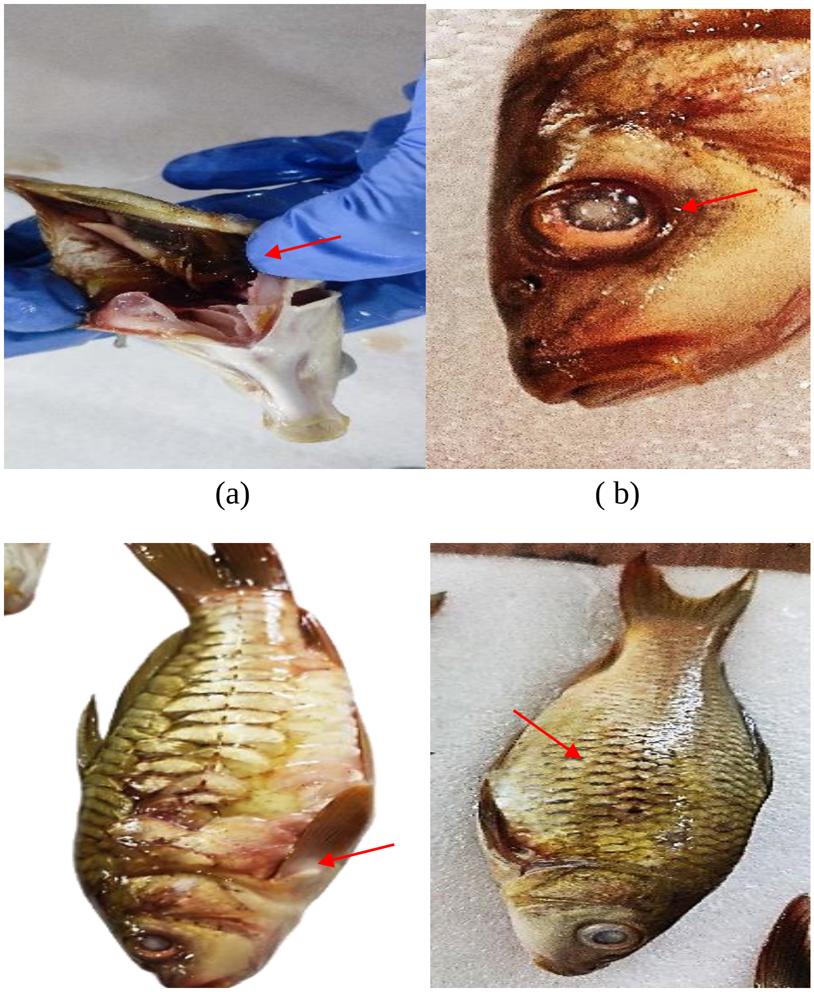
The infected group's clinical symptoms at the fifth day post infection included profuse mucus discharges all over the external body surface, haemorrhages at the bases of the fins, fin and tail rot, exophthalmia and ascites with a faintly protruding crimson vent. Kidney and hepatic congestion was detected in the postmortem lesions. On the other hand, fish belonging to the control negative groups did not exhibit any symptoms or have any huge fatalities (C) (Fig. 3).

3.4 Body weight and length of the infected and control fish

Tables (1, 2) show that there is a significant growth in length and weight of the controlled fish, while infected fish had no significant growth on the 5th day and at later time intervals of experiment.

Table 1. Comparison in length between infected and controlled fishes

S.No	Time interval (in days)	Length case Mean± S.D	Length Control Mean ± S.D	SE Mean I	SE Mean C	*P*- value
1	5	15.12 ±1.14	15.14 ±1.15	0.36	0.36	**>0.05**
2	15	15.12 ±1.16	15.25 ±1.08	0.37	0.34	**<0.05**
3	25	15.13± 1.17	16.44± 0.93	0.37	0.29	
4	35	15.13 ±1.17	16.92± 0.80	0.37	0.25	



(a) (b)

c) d)

Fig. 3. Infection caused by *Pseudomonas* sp. in experimental fishes showing:
a) Friable; b) Eye cataract; c) Red spots and fin erosion, and d) Abdominal distension

Table 2. Comparison in weight between infected and controlled fishes

S.No	Time interval (in days)	Weight case Mean ± S.D	Weight Control Mean ±S.D	SE Mean I	SE Mean C	*P*- value
1	5	24.15 ±1.45	26.58 ±3.07	0.46	0.97	**<0.05**
2	15	22.98 ±2.00	27.28 ±3.37	0.63	1.1	
3	25	22.21 ±1.95	28.18 ±3.71	0.62	1.2	
4	35	21.13 ±2.19	28.96 ± 3.69	0.69	1.2	

Tables (3 & 4) indicate that r^2 showed negative correlation. In the LW relationship, value of exponent b provides information on fish growth: b = 3 is positive allometric.

Table 3. Estimated parameters in the length-weight relationship of the infected fish through regression analysis

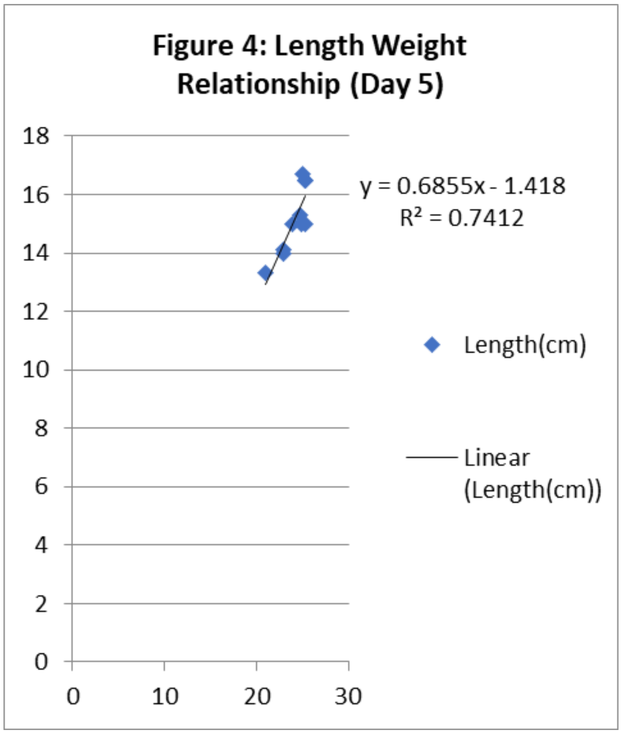
S.No	**Time interval (in days)**	**ln a**	**b**	R^2	**K**
1	5	–1.418	0.68	0.74	0.7
2	15	3.29	0.51	0.78	0.7
3	25	4.52	0.47	0.63	0.6
4	35	7.56	0.36	0.45	0.6

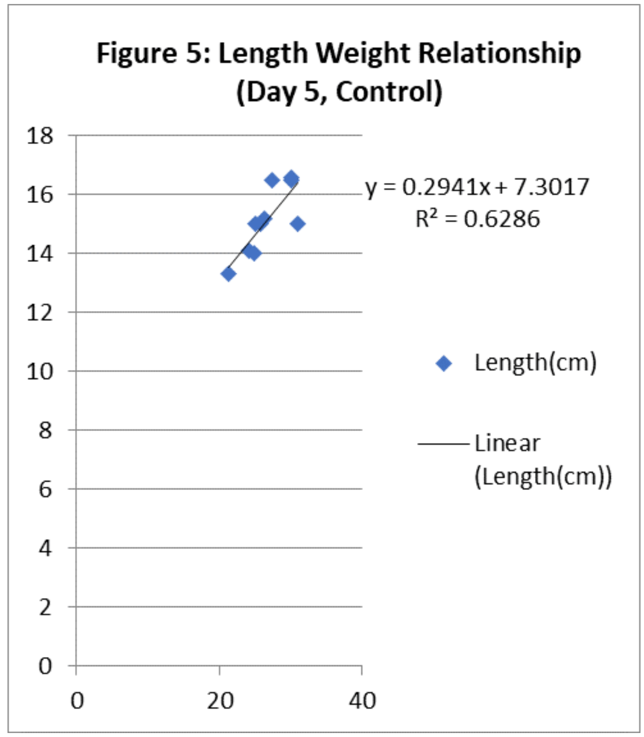
Table 4. Estimated parameters in the length-weight relationship of the controlled fish through regression analysis

S.No	**Time interval (in days)**	**ln a**	**b**	R^2	**K**
1	5	7.30	0.29	0.63	0.8
2	15	8.44	0.25	0.59	0.8
3	25	11.16	0.18	0.56	0.6
4	35	12.14	0.16	0.58	0.6

The fish becomes slim as it gets longer, gaining length faster than weight. Because the value of their b is less than 3, they were classified as negative allometric (Figs. 4- 11).

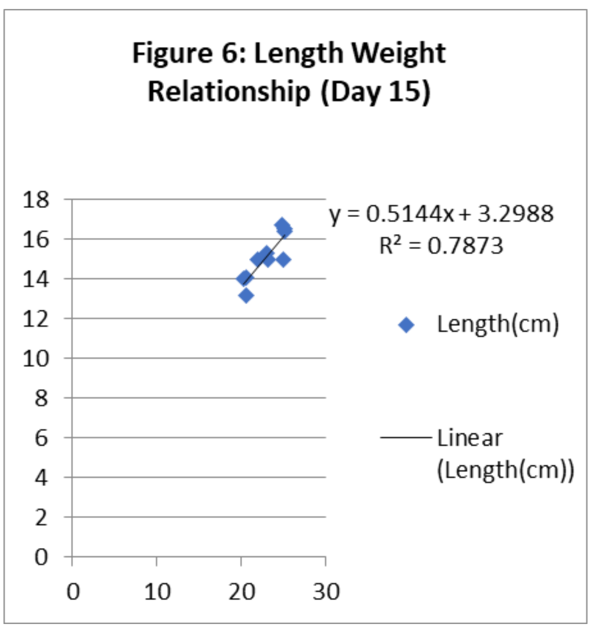
Other than that, it was interesting to find that the CFs from infected populations were less than 1.0, as shown in Tables (3 & 4), suggesting that fish population have not achieved their expected growth. The reason might be the artificially challenge of pathogenic bacteria in fishes of aquarium.

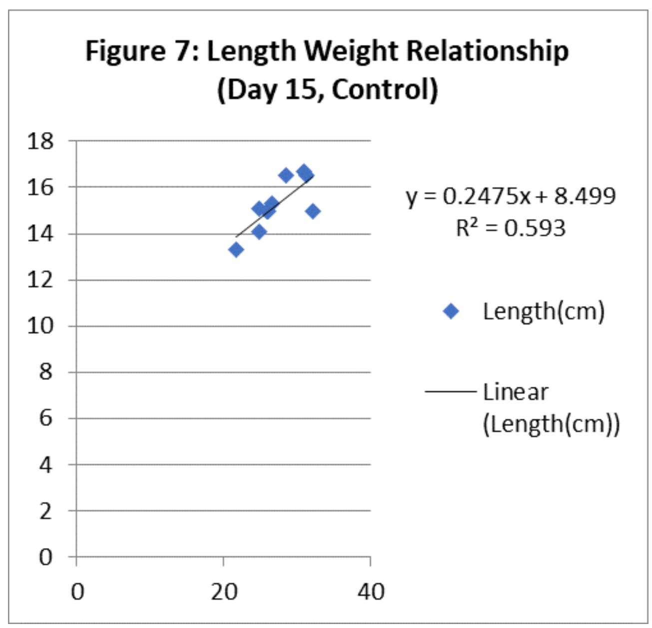




(a) **(b)**

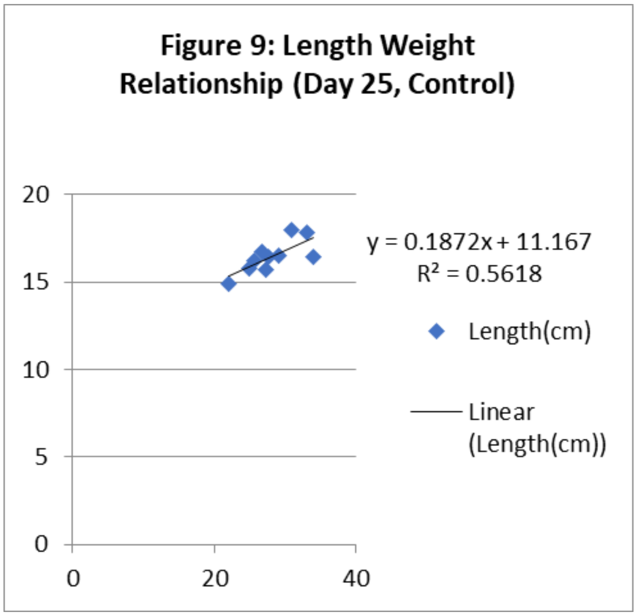
Fig. 4 and Fig. 5. Length weight equation graph of infected (a) and controlled (b) fish on day 5

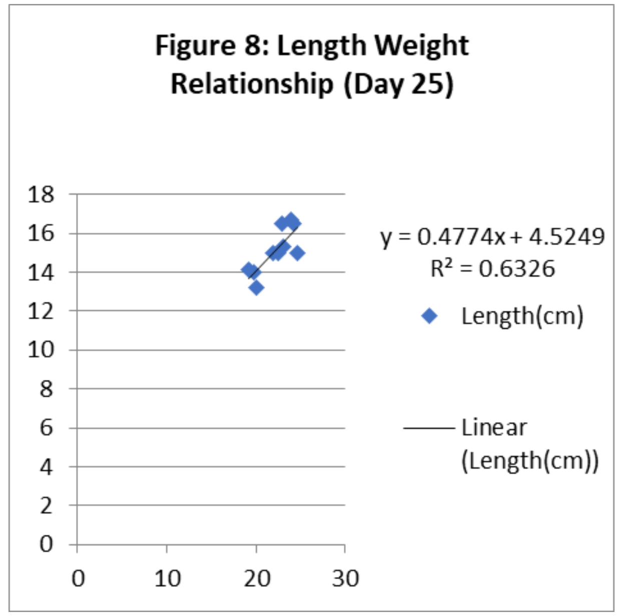




(c) **(d)**

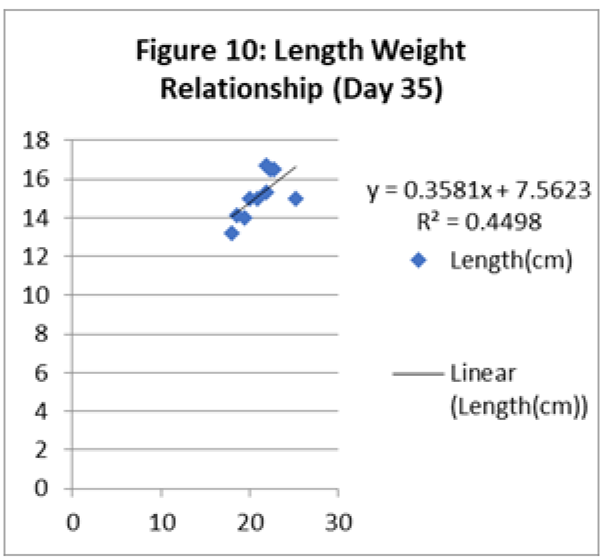
Fig. 6 and Fig.7. Length weight equation graph of infected (c) and controlled (d) fish on day 15

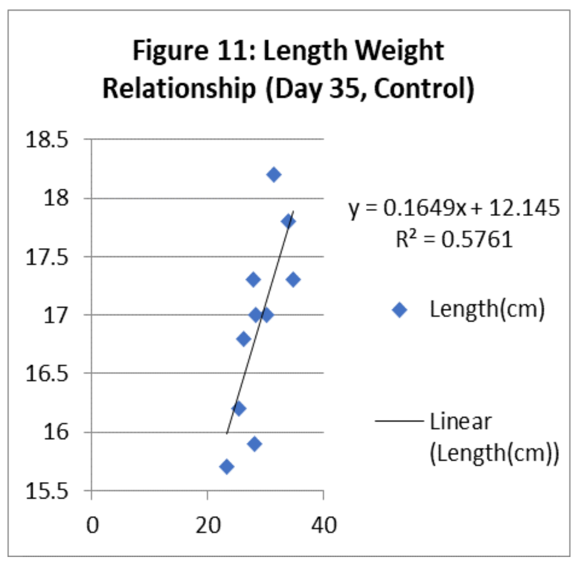




(e) (f)

Fig. 8 and Fig. 9 : Length weight equation graph of infected (e) and controlled (f) fish at Day 25





(g) (h)

Fig. 10 and Fig. 11. Length weight equation graph of infected (g) and controlled (h) fish

3.5 Alteration in hematological parameters

Bacterial infection significantly affected the hemoglobin (Hb) and total erythrocyte count (TEC), with highest values in control fishes and lowest in infected ones (Table 5). Infected fish showed slight decrease on day 10 and significant decrease on day 30, compared to control. The total leukocyte count (TLC) was significantly higher in infected one than the control on day 30.

Hematological parameter	Control MEAN ± S.D	IP $P_{S\ 10}$ MEAN ± S.D	IP $P_{S\ 20}$ MEAN ± S.D	IP $P_{S\ 30}$ MEAN ± S.D
WBC (10^4 mm^{-3})	3.15±0.14	2.75 ±0.34	3.73 ±0.29	4.71±0.76
Hematocrit %	29.69±0.93	25±1.03	21±1.20	16±0.86
Erythrocyte (10^6 mm^{-3})	2.31±0.16	1.73 ± 0.21	1.29± 0.13	1.21±30
Hemoglobin	10.37±0.61	5.76 ± 0.41	5.60± 0.46	5.13± 0.50
MCV	129.69±1.09	144.50 ±0.50	162.79±0.32	132.23 ±0.50
MCH	44.89±1.70	33.29± 1.10	43.41±1.24	42.39 ±0.40
MCHC	3.49±1.26	0.23 ±0.42	0.26±0.20	0.32 ±0.25

Table 5: *C. carpi* changes in hematological parameters (mean ± SD; n = 0) in infected (I) against control (C) fish infected with *Pseudomonas* (Ps) through Intraperitoneal Injection (IP). White blood cell (WBC: 10^4 mm^{-3}), red blood cells (RBC: 10^6 mm^{-3}), hemoglobin (Hb: g/dl), Hct (%) were obtained from 10, 20 and 30 days

DISCUSSION

The primary global barrier to the aquaculture business is bacterial illness. The results of recent experiments demonstrated that *Pseudomonas* sp. caused severe infection in common carp fish. The most prominent pathogenic bacteria for freshwater farmed fish have likewise been identified as *Pseudomonas* **(Thomas *et al.*, 2014)**.

Fish with a lower 'b' value (<1) have negative allometric growth **(Tesch, 1971; Ayoade & Ikulala, 2007)**. The form and fatness of the species, as well as physical parameters like as temperature, salinity, diet, sex, and maturation stage may influence b values **(Pauly, 1984; Sparre & Venema, 1992; Wooten, 1998; Sarkar *et al.*, 2013)**.

It has been discovered that infected fish are easier to catch than healthy ones. Monitoring fish behavior is a very effective method that is frequently used in the aquaculture industry to determine the health of the fish. In the fish farming industry, they are regarded as a maker to monitor illness conditions or environmental stress **(Nandi *et al.*, 2016)**.

The infected fish showed low feed intake rate in comparison to uninfected ones. The infected fish had haemorrhages, loosening scales, skin lesions, erosions at the fins and copious mucous discharge as clinical signs. Hemorrhage was seen in the gill, liver, kidneys and all vital organs. This research supported the findings of **Khalil *et al.* (2010)** among cultured *Oreochromis niloticus* and **Panda *et al.* (2012)** in infected *Labeo bata***.** Gills are the principal respiratory organs, and all metabolic pathways rely on the gills' effectiveness for energy delivery. Damage to these important organs sets off a cascade of events that leads to respiratory distress **(Magare & Patil, 2000)**. Infected fish have a noticeable mucus layer production over the gill lamellae. Mucus secretion across the gill results in oxygen diffusion, which may eventually diminishes the fish's oxygen absorption **(David *et al.* 2002).** As a result, behavioral changes might be caused by bacterial infection.

Diverse researchers have acquired an interest in the study of hematological and serum biochemical fish blood parameters in recent years to establish baseline values of fish species. Fish wellness is assessed using hematological measures because these blood indices reliably identify chronic complaints, deficits and metabolic disorders **(Bahmani *et al.*, 2001; Fazio *et al.*, 2013; Sheikh & Ahmed, 2019)**. Therefore, determining the hematological reference values provides early information on the assessment of fish health status **(Pradhan *et al.*, 2012)**.

Our findings are consistent with those of other researchers who conducted their studies on a variety of normal fish species, including *Tor putitora* **(Kapila *et al.*, 2000)**, *Clarias batrachus* **(Acharya & Mohanty, 2014)**, *Cirrhinus mrigala* **(Pradhan *et al.*, 2014)**, *Barilius bendelisis* **(Sharma *et al.*, 2017)**, *Hypophthalmichthys molitrix* **(Ahmed *et al.*, 2019)** and *S. plagiostomus* **(Sheikh & Ahmed, 2019)**. Whitefish growth is affected by eye fluke infection according to **Karvonen and Seppa la (2008)** research (*Coregonus lavaretus).* Fish used in experiments that were not infected with eye fluke grew more quickly than those that were infected. For final body length and weight, the fish in the control group were better.

CONCLUSION

The research demonstrated how *Pseudomonas* sp. influences the host fish's growth, hematological and behavioral factors. Significant alterations in the cultured host fish; however, have shown bacterial infection. The fact that the CFs from infected populations were less than 1.0 indicates that the fish population has not increased as anticipated. We may infer from the experiment that the *Pseudomonas* bacterium is a harmful one that harms the host fishes. Additionally, it reduced the host fish's life span and normal development. The findings demonstrated that when the count of WBC rises, the hematological parameters—namely, Hb, RBC and PCV—show a substantial drop in count with regard to time interval of infection. Therefore, the most significant findings are those regarding the delay of the host fish's normal development by the bacterial infection, in accordance to its hematological parameters and behavioral attitudes.

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