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Effect of stress conditions on condition factor and growth of farmed Rohu (*Labeo rohita*)

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Abstract
To investigate the effects of stressors on condition factor and specific growth rate in farmed *Labeo rohita*, 60 fingerlings were subjected to experimentation in laboratory aquaria after being acclimatized. Specimens were divided into control, starvation stress and double stress (pH = 8 and starvation) groups with 20 individuals in each group. Total length (L) and total weight (W) of the fishes was measured after 12, 24, 36 and 48 days. Condition factor (K) was calculated by using formula, $K = (W/L^3) \times 100$. % Specific growth rate was calculated by using formula, $\%SGR = (\log e\ W_2 - \log e\ W_1/T) \times 100$; where, T = Duration of experiment. The data was subjected to one way ANOVA for comparison between and within groups. There was considerable decrease in condition factor, though statistically non-significant, in starvation and double stress groups compared to control group after 12 days; however, this decrease was significant ($P<0.05$) after 48 days. Within group comparison revealed no significant effect of stressors on condition factor. A considerable decline in growth rate/de-growth was recorded in all three groups, comparatively more in magnitude in stress groups compared to the control. Within group comparison revealed a significant effect ($P<0.01$) of stress in starvation group, but statistically no significant effect in double stress group. It is concluded that stress conditions affect growth of *Labeo rohita* at early stages.

Keywords: Starvation, stress response, specific growth rate, condition factor, *Labeo rohita*

1. Introduction
Condition factor compares the wellbeing of a fish and is based on the hypothesis that heavier fish of a given length are in better condition [1]. It is a useful index for the monitoring of feeding intensity, age, and growth rates in fish [2]. Environmental factors may cause spatial and temporal differences in condition factor of fish [3, 4]. Growth is the change in size (Length, weight) either of organism's whole body or of it various tissues [5] and is a good indicator of the health of an individual [6]. Growth rates in fish may be highly variable and in many cases appear to be limited by food availability [7].
Stress causes a wide variety of physiological responses in fish [8, 9]. Severe or long lasting stressors lead to decreased growth, increased metabolic exhaustion and disease incidenceand possible mortality [9, 10]. Many fish undergo period of starvation e.g. during wintering, spawning, migration or when local food abundance diminishes [5]. Studies on fish starvation are important for better understanding of growth biology of fish [5]. The decrease in body weight of fish has been observed during starvation periods in *Cyprinus carpio* [11], *Dicentrarchus labrax* and *Pagellus bogaraveo* [12] and reduced levels of thyroid hormones, glucose and glycogen in Nile Tilapia [13].
Studies on effects of pH on growth of fish have become important due to increasing acidification caused by industrialization and urban emission of oxides [14]. The main effect of low pH on the fish seems to be an inhibition of oxygen uptake at the gills [15], whereas high pH may cause higher levels of unionized ammonia resulting in increased toxicity [16].
Successful management of fisheries requires that the effects of environmental stressors should be predicted [17]. *Labeo rohita* is a representative of the major carps of Indo-Pakistan sub-continent [18], and is commercially important due to its taste and consumer preference [19]. There are several effects of starvation stress and pH stress on growth of fish in pond condition. However, it is difficult to measure such effects in natural habitat. Therefore, present research work aimed to study the effect of stressors like starvation and pH on condition factor and specific growth rate of *Labeo rohita* in laboratory conditions.

2. Materials and Methods

Sixty specimens (fingerlings) of farmed Rohu were utilized for the experiment. The specimens were obtained from Qadria Fish Hatchery, Multan (30°29′N 60°88′E). The fishes were acclimatized to laboratory conditions in glass aquaria (36"×12"×5") for duration of 20 days. Meanwhile they were regularly fed on commercial fish diet. The experiment was conducted for a period of 48 days. The fish were divided into three groups i.e. control, starvation and double stress (pH = 8 and starvation), each consisting of 20 individuals. The control group was continuously fed throughout the experimental period. The starvation group was kept under starvation stress at neutral pH and the double stress group was kept under starvation at pH 8. The pH was maintained at 8 by using lime water (calcium hydroxide) and monitored from time to time with the help of pH meter (3071, Jenway). The aquaria were regularly cleaned after every four days by partial replacement of water.

Measurements for the calculation of condition factor and specific growth rate were taken after every 12 days. Total length of fishes was measured to nearest 0.01 cm using Perspex measuring tray fitted millimeter ruler. All measurements were made from the tip of maxilla to the largest caudal fin ray. Fish were weighed on an electronic digital balance; MP-3000 (Ohyo-Japan) to the nearest 0.01g. The condition factor (K) was calculated by using the formula: $K = (W/L^3) \times 100$; Where, W = weight and L = length [20, 21]. % Specific growth rate (%SGR) in terms of wet body weight was determined by using the formula: $\%SGR = (\log e\ W_2 - \log e\ W_1/T) \times 100$; Where, W_1 = Initial wet body weight, W_2 = Final wet body weight and T = Duration of experiment [14].

The data obtained was subjected to one way ANOVA, after log transformation, to compare the effects of starvation and double stress on condition factor value and % specific growth rate of fish. The percent increase/decrease in condition factor and specific growth rate was also calculated to estimate effect of starvation stress and double stress on growth of *Labeo rohita*.

3. Results

Comparison between control, starvation and double stress groups suggested that there was no significant effect of starvation and double stress on condition factor after 12, 24 and 36 days, however, a significant effect (df = 2, 9; F = 5.68; $P<0.05$) was observed after 48 days (Table 1). Both the starvation and double stress groups demonstrated considerable percentage of decrease in condition factor value after 12, 24 and 48 days compared to control group (Table 1). Within comparison of each group suggested that there was no significant effect ($P>0.05$) of starvation and double stress on condition factor, however, a significant effect (df = 3, 13; F = 4.54; $p<0.05$) was observed within the control group (Table 1). There was an overall considerable percentage decrease in condition factor value in control group with increasing number of days of experiment. While no considerable decrease was observed in starvation and double stress group with increasing number of days of experiment except double stress group showed a decrease of 20% after 48 days. However, a decrease in condition factor of 35% in starvation group and 41% in double stress group was revealed compared to initial control group value.

Comparison between control, starvation and double stress groups suggested that there was no significant effect of starvation and double stress on % specific growth rate after 12, 24, 36 and 48 days (Table 2). There was considerable decrease in % specific growth rate (de-growth), varying in magnitude, in control as well as stress groups that continued up to end of the experiment. Initial de-growth at 24 days was greater in magnitude in stress groups in comparison to control group. There was 70% de-growth in starvation group and 50% in double stress group after 48 days compared to initial de-growth observed in control group. Within comparison of each group suggested that there was a significant effect (df = 3, 7; F = 9.21; $P<0.01$) of starvation stress on % specific growth rate. However, there was no significant effect on % specific growth rate in control and double stress group (Table 2). There was an overall increase in de-growth in starvation and double stress group being maximum after 36 days. However, it started decreasing after 48 days observations in both groups. It is evident that the growth recovery response in starvation group and double stress group is more compared to control group. There was a steady decrease in de-growth in starvation group, however, in double stress group, no definite trend was recorded.

Table 1: Comparison of effect of stress (starvation) and double stress (pH + starvation) on condition factor (K) in *Labeo rohita*.

Treatments	Control	Starvation	Double Stress	£P value
12 days	1.04±0.24	0.69±0.01	0.74±0.13	0.07
24 days	0.88±0.15	0.77±0.09	0.74±0.05	0.30
36 days	0.70±0.16	0.69±0.01	0.73±0.04	0.86
48 days	0.75±0.04	0.66±0.09	0.59±0.03	0.02*
≠P value	0.02*	0.28	0.56	

£ = P values for one way ANOVA for comparison of condition factor values between groups
≠ = P values for one way ANOVA for comparison of condition factor values within groups

Table 2: Comparison of effect of stress (starvation) and double stress (pH + starvation) on% specific growth rate (%SGR) in *Labeo rohita*.

Treatments	Control	Starvation	Double Stress	£P value
12 days	- 1.26±0.92	-1.67±0.80	- 1.62±0.88	0.82
24 days	- 0.73±0.57	- 0.82±0.19	- 0.49±0.36	0.62
36 days	- 0.31±0.70	- 0.68±0.12	- 0.59±0.17	0.62
48 days	- 0.40±0.16	- 0.38±0.21	- 0.63±0.15	0.57
≠P value	0.41	0.002*	0.22	

£ = P values for one way ANOVA for comparison of% specific growth rate between groups
≠ = P values for one way ANOVA for comparison of% specific growth rate within groups

4. Discussion

The results of present study demonstrated a decrease in condition factor in starved and double stressed fish compared to control (Table 1). A number of factors including sex, seasons, environmental conditions, stress and availability of food etc. affect the condition of fish. Food restriction for a period of 12-week in *Cyprinus carpio* had a significant impact on condition factor that decreased to 22.5% after 10 weeks [22]. In sea bass and in black spot sea bream, the condition factor (*K*) values showed a decreasing trend (4.41 and 5.88%, respectively) after 31 days of starvation compared to the initial value [12]. Even in alternate starvation and feed cycles for 10 days, a significant decrease of 9.3% in condition factor in comparison to control in *Cirrhinus mrigala* has been reported [23]. In another study, 8.5% decrease was observed in condition factor in alternate starvation and feed cycles for 10 days in *Labeo rohita*, though the decrease was not significant [24].

In the present study, an initial de-growth was observed in stress groups as well as in control group which seems to be the effect of stress imposed by confined environment of aquaria which may cause a loss of appetite in fish. This is in agreement with already published information which reported that *Catla catla* [25] and bighead carp [26] did not show any increase in growth rate in control groups. The initial de-growth in control start decreasing gradually after 12 days till 48 days, thus demonstrating a steady de-growth pattern in control group. It seems that the control fish do not prefer artificial feed in experimental aquaria. It may consume just its maintenance ration [10].

In *Cyprinus carpio*, during the 12-week-long period, food restriction had a significant impact on hepatosomatic index and viscerosomatic index which reduced significantly [22]. Starvation of 21 days resulted in a 17% decrease in body mass of black carp [27]. In both sea bass and black spot sea bream, weight decreased in the starved group, from the beginning to the end of the experiment, although not significant. However, in sea bass significant decrease was recorded in starved group with a weight loss of 18.39% after 31 days compared to feed group [12]. Even in alternate starvation and feed cycles for 10 days, negative specific growth rate has been observed in *Cirrhinus mrigala*. The specific growth rate (% g day^{-1}) decreased significantly (184%) in comparison to control in *Cirrhinus mrigala*. There was a sharp decrease in specific growth rate with increase in length of starvation [23]. Similarly, in another study, a negative specific growth rate was observed in alternate starvation and feed cycles for 10 days in *Labeo rohita*. The specific growth rate significantly decreased (183.6%) in comparison to control in *Labeo rohita* [24].

In the present study it was observed that during starvation stress and double stress fish loses its body weight. The decrease in body weight was greater in double stress group than in starvation group. In fish, at lower pH levels, the voluntary food consumption declines and there is a corresponding decline in growth rates [5]. Fish ponds with higher pH may develop relatively higher levels of un-ionized ammonia, which is toxic [28]. Generally at pH 7 only less than 1% of the total ammonia is in the toxic un-ionized form, at pH 8 about 5 to 9%, at pH 9 about 30 to 50%, while at pH 10 is about 80 to 90%. The first mortalities from prolonged exposure to toxic ammonia begin at concentration as low as 0.2mg/L and this un-ionized form of ammonia had been reported to depress appetite of tilapia at concentration as low as 0.08mg/L [16].

It is concluded that starvation stress significantly affected growth rate of *Labeo rohita*. Double stress also considerably decreased growth rate of *Labeo rohita*. There was an initial decline of growth reflected as de-growth, not only in starvation and double stress group but also in control group. Afterwards growth rates gradually increased with an increase in number of days. Variations in growth response in starvation and double stress group were more compared to control group.

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