

## Research Article

# Growth responses of carnivorous fish species under the chronic stress of water-borne copper

Asif S.<sup>1</sup>; Javed M.<sup>1</sup>; Abbas S.<sup>1\*</sup>; Ambreen F.<sup>2</sup>; Iqbal S.<sup>1</sup>

Received: March 2020

Accepted: October 2020

### Abstract

This study aimed to investigate the growth performance of three length groups (50, 100 and 150mm) of carnivorous fish species, *Channa marulius* and *Mystus seenghala*, under sub-lethal ( $1/3^{\text{rd}}$  of  $LC_{50}$ ) exposure of water-borne copper, in terms of increase/decrease in average wet weights (g) and total lengths (mm), condition factor, feed intake (g) and feed conversion efficiency (%). A group of 10 fish of each species and length group, with three replications, was separately exposed to water-borne copper, for 120 days, at constant conditions of water temperature (28°C), pH (8) and total hardness (250 mg L<sup>-1</sup>). Weekly data on all growth parameters of fish were collected and analyzed statistically. Results showed that treated fish species exhibited significantly lower average wet weight and total length increments than control fish that followed the order: *C. marulius*>*M. seenghala*. Among three length groups of both fish species, 150 mm length group exhibited significantly higher growth in terms of average wet weight and total length increments than 100 mm and 50 mm length groups suggesting that younger fish became more susceptible to copper stress hence showed lesser growth than older fish. The “K” values computed were significantly higher for treated than the control fish. Copper treated fish exhibited significantly lower feed intake than control fish. However, among three length groups of fish, order of feed intake was 150 mm>100 mm>50 mm. *C. marulius* exhibited significantly higher FCE as compared to *M. seenghala*. Overall, it was concluded that sub-lethal copper exposure caused significant reduction in growth of carnivorous fish.

**Keywords:** Copper, Sub-lethal exposure, Carnivorous fish, Growth performance

1- Department of Zoology, Faculty of Life Sciences, Gulab Devi Educational Complex, Lahore, Pakistan

2- Department of Zoology, Government College Women University, Madina Town, Faisalabad, Pakistan

\*Corresponding author's Email: sidraabbas2012@hotmail.com

## Introduction

The contamination of freshwater ecosystems with a wide variety of toxicants has become a major issue worldwide over the past several decades (Vutukuru, 2005; Bae *et al.*, 2020; Moon *et al.*, 2020). The causative agents disturbing the natural freshwater ecosystems include metal-based fertilizers, industrial and sewage wastes, agricultural runoff, acidic rainfall as well as soil leaching (Prasath and Airvoli, 2008; Atique *et al.*, 2020; Khanom *et al.*, 2020). Industrial effluents are the main source of heavy metals pollution of receiving ecosystems i.e. soil, sediments and water, and ultimately posing serious threats to the indigenous fish fauna of Pakistan (Rauf *et al.*, 2009). Metals have a non-biodegradable property while the rest of the water contaminants can be biodegraded into the simplest and less toxic forms over time (Wepener *et al.*, 2001). However, metals may transform into more stable, more toxic and complex forms (Yang and Rose, 2003).

Among all the aquatic toxicants, metals are considered to be the most toxic due to their stability, long persistence and potential harms during biomagnification in the aquatic food chains (Moorthikumar and Muthulingam, 2010; Saeed *et al.*, 2020). Zinc, copper and selenium are among the trace elements which are primarily required for normal metabolism, while concentrations above the permissible limits would cause lethal effects to the organisms. Copper, cadmium, silver, manganese,

chromium, nickel, lead, mercury and zinc are principally studied as indicators to assess the pollution status of aquatic ecosystems as they get easily dissolved in the aqueous medium (Sundarraaj *et al.*, 2014). The heavy metals are taken up by the living organisms, accumulated in different organs that can lead to reducing fish growth or may cause mortality (Govind and Madhuri, 2014).

To evaluate the pollution level of an aquatic ecosystem, fish is ideally used because it is present at the top of the aquatic food chain. As human diet, fish may cause hazardous effects to the human due to its habitat pollution as fish could not escape from its environment (Ahmed *et al.*, 2016). The biological methods are applied for the estimation of toxic effects of contaminants on the sensitivity of various test organisms towards their physiological, morphological, and behavioral indices (Parasuraman, 2011). The condition factor (K) of a fish is considered to be the most important biological parameter that gives information about the health status of fish and its community (Richter, 2007). Feed intake undoubtedly influences the growth rates of fish as there is an intricate relationship among growth, feeding and other factors of fitness (Handeland *et al.*, 2008). Feed conversion efficiency (FCE) is the foremost variable in the aquaculture enterprises that act as a limiting factor to assess the growth responses of fish (Doupe and Lymbery, 2003).

Like all the other essential trace metals, copper is also required for normal body

functions and growth of all aquatic organisms. It is important for enzymatic activity and plays a significant role in cellular processes like oxido-reductive reactions. Copper sulfate is commercially used in drugs, pesticides and as a constituent of electric appliances (Gan *et al.*, 2013). It is widely used to control algal, fungal and bacterial diseases of fruit orchards, vegetables and crops due to its algacide and fungicide properties (Atamanalp *et al.*, 2008). The copper toxicity is linked with its ionic form i.e.  $\text{Cu}^{+2}$ . The higher concentration of metals in the aquatic medium resulted in the formation of free radicals in the body of aquatic organisms that can cause toxic effects and certain chromosomal aberrations (Ambreen and Javed, 2016; Ashraf *et al.*, 2018). Presently, the chronic toxicity of copper induces ill-effects on the aquatic organisms and ultimately disturbs the feeding rate of species resulting in the fluctuation in the food pyramid (Bogdanova *et al.*, 2002).

*Channa marulius* and *Mystus seenghala* are the carnivorous fish species belong to family "channidae" and "bagridae", respectively (Bhatti *et al.*, 2013). They are on the top of the aquatic food chain and are mostly found in riverine systems of Punjab, Khyber Pakhtunkhwa, Balochistan, and Sindh. In Pakistan, its commercial value is very high (Rafique and Khan, 2012). They are known for their higher protein and lower fat content than tilapia and carps. However, the excessive discharge of untreated wastewater has

deteriorated the quality of water that can affect the growth performance of carnivorous fish (Skouras *et al.*, 2003). Therefore, the present research project was conducted to investigate the growth responses of two carnivorous fish species viz. *C. marulius* and *M. seenghala* exposed to sub-lethal ( $1/3^{\text{rd}}$  of  $\text{LC}_{50}$ ) concentrations of water-borne copper for 120 days.

### Materials and methods

This research was carried out at Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. The carnivorous fish species viz. *C. marulius* and *M. seenghala* were brought from Head Qadirabad situated at river Chenab in the Punjab region. They were kept in cemented tanks for 10 days to acclimatize under laboratory conditions. During the acclimatization period, pelleted feed was supplied to the fish. After acclimatization, each group of fish species with similar lengths and weights was selected and divided into two groups ( $n=10$ ) viz. "treated" and "control". The both groups were separately transferred into the glass aquaria. Stock solutions of desired concentrations were prepared by dissolving  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in deionized water. The treated fish group was exposed to  $1/3^{\text{rd}}$  of  $\text{LC}_{50}$  copper concentrations (Table 1) as determined by Javed (2015) in 35L water capacity glass aquaria at constant temperature ( $28^\circ\text{C}$ ), pH (8) and total hardness ( $250\text{mg L}^{-1}$ ), for 120 days.

**Table 1: Sub-lethal ( $1/3^{\text{rd}}$  of  $LC_{50}$ ) concentrations of copper for the three length groups of carnivorous fish species.**

| Length groups | Fish species        | Sub-lethal concentrations ( $\text{mgL}^{-1}$ ) |
|---------------|---------------------|---|
| 50mm          | <i>C. marulius</i>  | $19.51 \pm 0.05$                                |
|               | <i>M. seenghala</i> | $4.68 \pm 0.02$                                 |
| 100mm         | <i>C. marulius</i>  | $22.81 \pm 0.07$                                |
|               | <i>M. seenghala</i> | $7.12 \pm 0.03$                                 |
| 150mm         | <i>C. marulius</i>  | $30.32 \pm 0.08$                                |
|               | <i>M. seenghala</i> | $8.75 \pm 0.03$                                 |

However, the other group (control) was kept in a metal free environment. Aeration was provided continuously to the fish during each growth trial.

The growth experiments were conducted with three replications for each test concentration for each fish species and length group. During the whole experimental period, fish were fed to satiation twice a day with the supplementary feed having  $3.50 \text{Kcalg}^{-1}$  digestible energy and 45% digestible protein. The growth parameters of 50, 100 and 150mm length groups of *Channa marulius* and *Mystus seenghala* under treated and control media were monitored on weekly basis in terms of increase/decrease in average wet weights and total lengths, condition

factor, feed intake, and feed conversion efficiency (Figs. 1 and 2).

The water quality characteristics viz. temperature, pH, carbon dioxide, dissolved oxygen, total hardness, and total ammonia contents have considerable effects on the copper toxicity. So, these parameters should be maintained during the trials. Water temperature and pH were recorded by electronic meter HANNA HI-8424 while dissolved oxygen by using the digital meter, HANNA HI-9146. However, total hardness, total ammonia, carbon dioxide, calcium, magnesium, sodium and potassium were analyzed by methods of American Public Health Association (APHA) (2012).

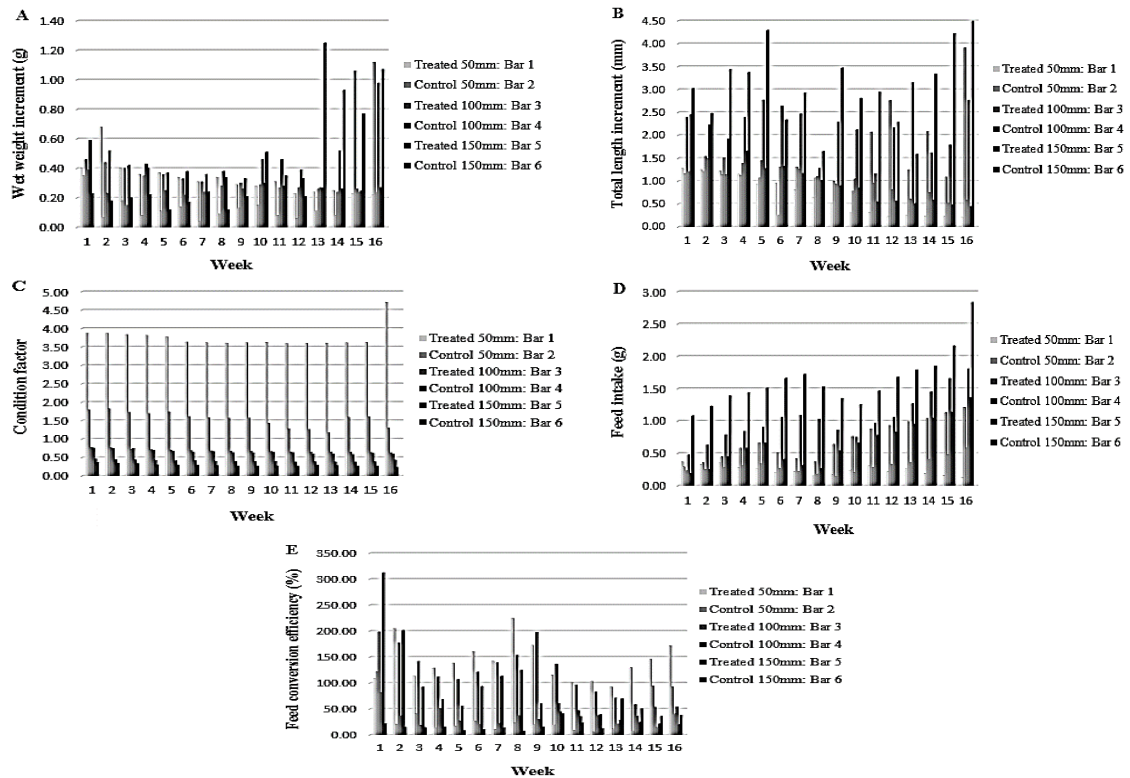


Figure 1: Weekly variations on wet weight increment (g) (A), total length increment (mm) (B), condition factor (C), feed intake (g) (D) and feed conversion efficiency (%) (E) of 50, 100 and 150mm copper treated and control *Channa marulius*.

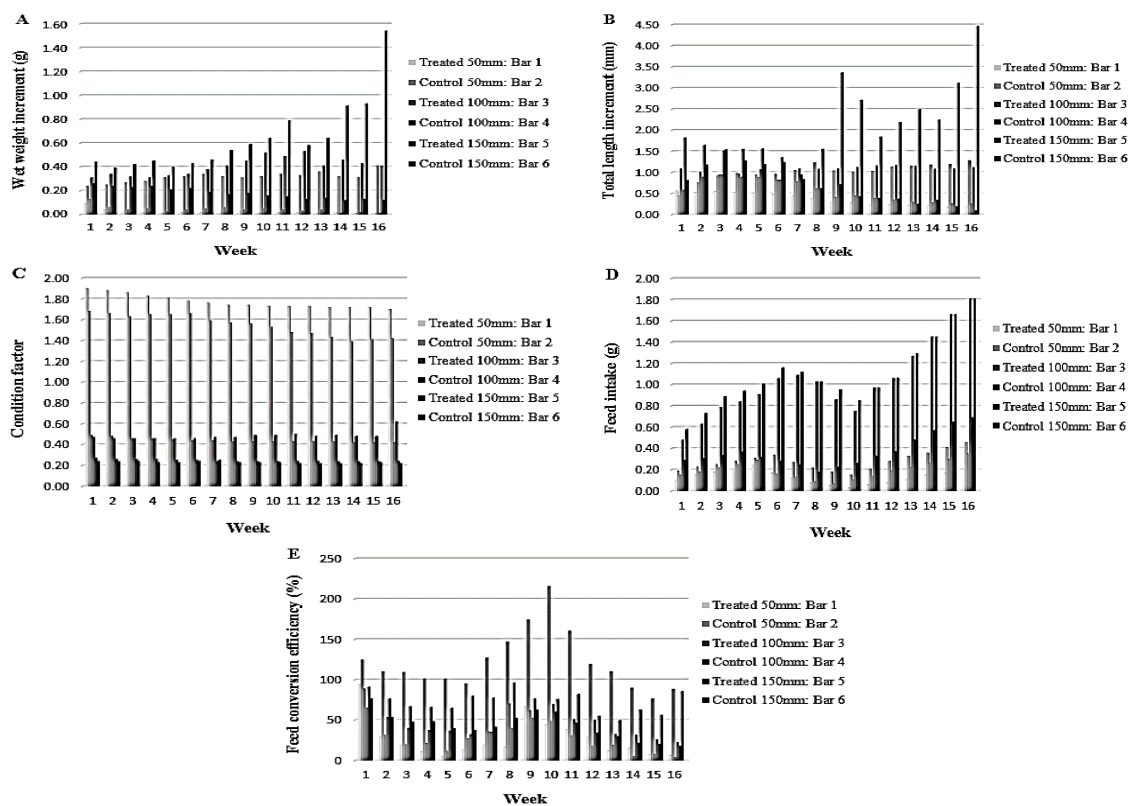


Figure 2: Weekly variations on wet weight increment (g) (A), total length increment (mm) (B), condition factor (C), feed intake (g) (D) and feed conversion efficiency (%) (E) of 50, 100 and 150mm copper treated and control *Mystus seenghala*.

### Statistical analyses

Three replications were used for each length group, treatment and fish species. The differences between various growth parameters (average wet weight (g) and total length (mm) increments, condition factor, feed intake (g), and feed conversion efficiency (%)) of 50, 100 and 150mm length groups of treated and control fish (*Channa marulius* and *Mystus seenghala*) were analyzed statistically through Factorial experiments under Randomized Complete Block Design (RCBD) by using Statistix<sup>8.1</sup> computer software. The means were compared for statistical differences by employing Tukey's/Student Newman-Keul test.

## Results

### Average wet weight increments

The experiments were performed to determine the sub-lethal copper exposure effects on the growth performance of 50, 100 and 150 mm length groups of two carnivorous fish species viz. *C. marulius* and *M. seenghala*. There existed statistically highly significant differences among the length groups, treatments and species regarding increase in average wet weights (Table 2).

Regarding overall means computed for increase in average wet weights of the three length groups of fish, the order found was 150mm>100mm>50mm. The treated fish species showed minimum increase in average wet weight than the control fish. Among the two fish species, *C. marulius* exhibited maximum increase in average wet weight as compared to the *M. seenghala*.

### Average total length increments

The analysis of variance on increase in average total lengths shows that there existed statistically highly significant differences among the length groups, treatments and species (Table 3).

Among the three length groups of fish, 50 mm length group showed lower increase in average total length than 100mm and 150 mm length groups. However, the copper treated *C. marulius* and *M. seenghala* exhibited lower average total length increments as compared to the control fish. The fish, *M. seenghala* revealed lower increase in average total length than that of *C. marulius* with the mean values of  $0.98 \pm 0.52$  and  $1.57 \pm 0.85$  mm, respectively.

**Table 2: Analysis of variance on increase in average wet weights (g±SD) of fish.**

| Length groups x Species    |                    |                     |                   |
|----------------------------|--------------------|---------------------|-------------------|
| Length groups              | Species            |                     | *Overall means±SD |
|                            | <i>C. marulius</i> | <i>M. seenghala</i> |                   |
| 50mm                       | 0.28±0.13 b        | 0.17±0.07 c         | 0.23±0.08 c       |
| 100mm                      | 0.34±0.14 ab       | 0.22±0.11 b         | 0.28±0.08 b       |
| 150mm                      | 0.39±0.16 a        | 0.41±0.17 a         | 0.40±0.01 a       |
| Overall means±SD           | 0.34±0.06 a        | 0.27±0.13 b         |                   |
| Length groups x Treatments |                    |                     |                   |
| Length groups              | Treatments         |                     |                   |
|                            | Treated            | Control             |                   |
| 50mm                       | 0.18±0.08 b        | 0.28±0.13 a         | 0.23±0.07 c       |
| 100mm                      | 0.18±0.08 b        | 0.39±0.16 a         | 0.28±0.14 b       |
| 150mm                      | 0.27±0.12 b        | 0.53±0.18 a         | 0.40±0.18 a       |
| Overall means±SD           | 0.21±0.05 b        | 0.40±0.13 a         |                   |
| Species x Treatments       |                    |                     |                   |
| Species                    | Treatments         |                     |                   |
|                            | Treated            | Control             |                   |
| <i>C. marulius</i>         | 0.33±0.13 a        | 0.34±0.14 b         | 0.34±0.01 a       |
| <i>M. seenghala</i>        | 0.08±0.05 b        | 0.45±0.17 a         | 0.27±0.26 b       |
| Overall means±SD           | 0.21±0.18 b        | 0.40±0.08 a         |                   |

Means with similar letters within a single row and \*column are statistically non-significant at  $p<0.05$ .

**Table 3: Analysis of variance on increase in average total lengths (mm±SD) of fish.**

| Length groups x Species    |                    |                     |                   |
|----------------------------|--------------------|---------------------|-------------------|
| Length groups              | Species            |                     | *Overall means±SD |
|                            | <i>C. marulius</i> | <i>M. seenghala</i> |                   |
| 50mm                       | 1.06±0.11 c        | 0.69±0.06 c         | 0.88±0.26 c       |
| 100mm                      | 1.58±0.15 b        | 0.82±0.08 b         | 1.20±0.54 b       |
| 150mm                      | 2.08±0.21 a        | 1.43±0.14 a         | 1.76±0.46 a       |
| Overall means±SD           | 1.57±0.51 a        | 0.98±0.40 b         |                   |
| Length groups x Treatments |                    |                     |                   |
| Length groups              | Treatments         |                     |                   |
|                            | Treated            | Control             |                   |
| 50mm                       | 0.51±0.05 b        | 1.24±0.12 a         | 0.88±0.52 c       |
| 100mm                      | 0.88±0.09 b        | 1.52±0.15 a         | 1.20±0.45 b       |
| 150mm                      | 0.99±0.10 b        | 2.52±0.25 a         | 1.76±1.08 a       |
| Overall means±SD           | 0.79±0.25 b        | 1.76±0.67 a         |                   |
| Species x Treatments       |                    |                     |                   |
| Species                    | Treatments         |                     |                   |
|                            | Treated            | Control             |                   |
| <i>C. marulius</i>         | 0.97±0.10 a        | 2.17±0.21 a         | 1.57±0.85 a       |
| <i>M. seenghala</i>        | 0.61±0.06 b        | 1.35±0.13 b         | 0.98±0.52 b       |
| Overall means±SD           | 0.79±0.25 b        | 1.76±0.58 a         |                   |

Means with similar letters within a single row and \*column are statistically non-significant at  $p<0.05$ .

### Condition factor

Table 4 shows analysis of variance on condition factor that varied significantly among the length groups, treatments and species. Regarding overall means of condition factor, the three length

groups followed the order: 50 mm>100 mm>150 mm. Highly significant differences were found between two treatments viz. copper treated and control with the mean values of  $1.22\pm1.34$  and  $0.79\pm0.66$ , respectively.

The differences between both the fish species were also found highly significant at  $p < 0.01$  for their condition factor. Among the two fish species, *C.*

*marulius* exhibited significantly higher condition factor values than the *M. seenghala*.

**Table 4: Analysis of variance on condition factor ( $\pm$ SD) of fish.**

| Length groups x Species |                    |                     |                         |
|-------------------------|--------------------|---------------------|-------------------------|
| Length groups           | Species            |                     | *Overall means $\pm$ SD |
|                         | <i>C. marulius</i> | <i>M. seenghala</i> |                         |
| 50mm                    | 2.64 $\pm$ 0.26 a  | 1.65 $\pm$ 0.16 a   | 2.15 $\pm$ 0.70 a       |
| 100mm                   | 0.67 $\pm$ 0.06 b  | 0.46 $\pm$ 0.05 b   | 0.57 $\pm$ 0.15 b       |
| 150mm                   | 0.35 $\pm$ 0.04 c  | 0.24 $\pm$ 0.02 c   | 0.30 $\pm$ 0.08 c       |
| Overall means $\pm$ SD  | 1.22 $\pm$ 1.24 a  | 0.78 $\pm$ 0.76 b   |                         |

| Length groups x Treatments |                   |                   |                   |
|----------------------------|-------------------|-------------------|-------------------|
| Length groups              | Treatments        |                   |                   |
|                            | Treated           | Control           |                   |
| 50mm                       | 2.76 $\pm$ 0.27 a | 1.53 $\pm$ 0.15 b | 2.15 $\pm$ 0.87 a |
| 100mm                      | 0.56 $\pm$ 0.06 a | 0.57 $\pm$ 0.07 a | 0.57 $\pm$ 0.01 b |
| 150mm                      | 0.33 $\pm$ 0.03 a | 0.26 $\pm$ 0.02 b | 0.30 $\pm$ 0.05 c |
| Overall means $\pm$ SD     | 1.22 $\pm$ 1.34 a | 0.79 $\pm$ 0.66 b |                   |

| Species x Treatments   |                   |                   |                   |
|------------------------|-------------------|-------------------|-------------------|
| Species                | Treatments        |                   |                   |
|                        | Treated           | Control           |                   |
| <i>C. marulius</i>     | 1.61 $\pm$ 0.16 a | 0.83 $\pm$ 0.08 a | 1.22 $\pm$ 0.55 a |
| <i>M. seenghala</i>    | 0.82 $\pm$ 0.08 b | 0.74 $\pm$ 0.07 b | 0.78 $\pm$ 0.06 b |
| Overall means $\pm$ SD | 1.22 $\pm$ 0.56 a | 0.79 $\pm$ 0.06 b |                   |

Means with similar letters within a single row and \*column are statistically non-significant at  $p < 0.05$ .

#### Feed intake

The analysis of variance on feed intake shows that there existed statistically highly significant differences among the length groups, treatments and species (Table 5).

Among the three length groups of *C. marulius* and *M. seenghala*, the 150 mm length group showed significantly higher feed intake, followed by that of 100 mm and 50 mm length groups. Among the treatments, the copper treated fish exhibited lower feed intake as compared to the control fish. *C. marulius* showed higher feed intake values than that of *M. seenghala*, among the two fish species.

#### Feed conversion efficiency

Table (6) shows that statistically highly significant variations were existed in the feed conversion efficiency, among all the length groups, treatments and species. The overall means showed that 50 mm length group of *C. marulius* and *M. seenghala* revealed better FCE than the other length groups of fish. However, there existed statistically non-significant variations in FCE of 100 mm and 150 mm fish length groups.



**Table 5: Analysis of variance on feed intake (g±SD) of two fish species.**

| <b>Length groups x Species</b>    |                    |                     |                          |
|-----------------------------------|--------------------|---------------------|--------------------------|
| <b>Length groups</b>              | <b>Species</b>     |                     | <b>*Overall means±SD</b> |
|                                   | <i>C. marulius</i> | <i>M. seenghala</i> |                          |
| <b>50mm</b>                       | 0.47±0.04 c        | 0.21±0.02 c         | <b>0.34±0.18 c</b>       |
| <b>100mm</b>                      | 0.67±0.07 b        | 0.62±0.06 b         | <b>0.65±0.04 b</b>       |
| <b>150mm</b>                      | 1.14±0.14 a        | 0.73±0.08 a         | <b>0.94±0.29 a</b>       |
| <b>Overall means±SD</b>           | <b>0.76±0.34 a</b> | <b>0.52±0.27 b</b>  |                          |
| <b>Length groups x Treatments</b> |                    |                     |                          |
| <b>Length groups</b>              | <b>Treatments</b>  |                     |                          |
|                                   | <b>Treated</b>     | <b>Control</b>      |                          |
| <b>50mm</b>                       | 0.19±0.01 b        | 0.49±0.04 a         | <b>0.34±0.21 c</b>       |
| <b>100mm</b>                      | 0.25±0.02 b        | 1.04±0.10 a         | <b>0.65±0.56 b</b>       |
| <b>150mm</b>                      | 0.51±0.05 b        | 1.36±0.13 a         | <b>0.94±0.60 a</b>       |
| <b>Overall means±SD</b>           | <b>0.32±0.17 b</b> | <b>0.96±0.44 a</b>  |                          |
| <b>Species x Treatments</b>       |                    |                     |                          |
| <b>Species</b>                    | <b>Treatments</b>  |                     |                          |
|                                   | <b>Treated</b>     | <b>Control</b>      |                          |
| <i>C. marulius</i>                | 0.40±0.05 a        | 1.12±0.12 a         | <b>0.76±0.51 a</b>       |
| <i>M. seenghala</i>               | 0.23±0.02 b        | 0.80±0.08 b         | <b>0.52±0.40 b</b>       |
| <b>Overall means±SD</b>           | <b>0.32±0.12 b</b> | <b>0.96±0.23 a</b>  |                          |

Means with similar letters within a single row and \*column are statistically non-significant at  $p<0.05$ .

**Table 6: Analysis of variance on feed conversion efficiency (%±SD) of fish.**

| <b>Length groups x Species</b>    |                      |                      |                          |
|-----------------------------------|----------------------|----------------------|--------------------------|
| <b>Length groups</b>              | <b>Species</b>       |                      | <b>*Overall means±SD</b> |
|                                   | <i>C. marulius</i>   | <i>M. seenghala</i>  |                          |
| <b>50mm</b>                       | 85.06±0.53 a         | 74.30±0.43 a         | <b>79.68±7.61 a</b>      |
| <b>100mm</b>                      | 63.13±0.37 b         | 36.82±0.25 c         | <b>49.98±18.60 b</b>     |
| <b>150mm</b>                      | 51.82±0.31 c         | 57.86±0.34 b         | <b>54.84±4.27 b</b>      |
| <b>Overall means±SD</b>           | <b>66.67±16.90 a</b> | <b>56.33±18.79 b</b> |                          |
| <b>Length groups x Treatments</b> |                      |                      |                          |
| <b>Length groups</b>              | <b>Treatments</b>    |                      |                          |
|                                   | <b>Treated</b>       | <b>Control</b>       |                          |
| <b>50mm</b>                       | 81.71±0.49 a         | 77.65±0.46 b         | <b>79.68±2.87 a</b>      |
| <b>100mm</b>                      | 72.56±0.41 a         | 27.40±0.22 b         | <b>49.98±31.93 b</b>     |
| <b>150mm</b>                      | 68.65±0.39 a         | 41.03±0.29 b         | <b>54.84±19.53 b</b>     |
| <b>Overall means±SD</b>           | <b>74.31±6.70 a</b>  | <b>48.69±25.99 b</b> |                          |
| <b>Species x Treatments</b>       |                      |                      |                          |
| <b>Species</b>                    | <b>Treatments</b>    |                      |                          |
|                                   | <b>Treated</b>       | <b>Control</b>       |                          |
| <i>C. marulius</i>                | 109.98±0.60 a        | 23.36±0.21 b         | <b>66.67±61.25 a</b>     |
| <i>M. seenghala</i>               | 38.63±0.26 b         | 74.02±0.42 a         | <b>56.33±25.02 b</b>     |
| <b>Overall means±SD</b>           | <b>74.31±50.45 a</b> | <b>48.69±35.82 b</b> |                          |

Means with similar letters within a single row and \*column are statistically non-significant at  $p<0.05$ .

The copper treated fish groups showed higher feed conversion efficiencies than the control fish groups. Regarding overall means, *C. marulius* exhibited higher values of FCE as compared to

*M. seenghala*, among the two fish species. However, in control media, *M. seenghala* showed better FCE as compared to the *C. marulius* with the

mean values of  $74.02 \pm 0.42$  and  $23.36 \pm 0.21\%$ , respectively.

## Discussion

Metals can cause adverse impacts on the fish immunity that can ultimately lead to increased disease susceptibility as well as decline in reproduction and growth capabilities (DeForest and Meyer, 2014). The exposure of sub-lethal concentration of metals could cause chronic stress leading to lesser growth in fish (Jadhav *et al.*, 2010). During present study, 150 mm length group gained significantly higher wet body weight, total lengths and feed intake as compared to the 100 mm and 50 mm length groups of fish. On the other hand, the condition factor and FCE values were significantly higher in 50 mm length groups. Length (age) group related toxicity of metals to the fish was also reported by Abbas *et al.* (2018). The present results are parallel to the findings of Subathra and Karuppasamy (2007) who concluded that fingerlings displayed reduced feeding and growth rates than the adult fish, *Mystus vittatus* when exposed to sub-lethal copper concentrations for duration of 60 days. Furthermore, there were significant differences in feed conversion efficiencies of two life stages of metal treated and control fish. Ansari *et al.* (2006) observed that copper toxicity to the fish (*Puntius chola*) decreased significantly with an increase in body length. The growth behaviours of 60, 90 and 120-day old fish age groups of two species (*Labeo rohita* and *Catla catla*) under sub-lethal copper exposure concentrations were

investigated by Ameer *et al.* (2013). They found that the 120-day metal stressed fish had significantly better wet weight increment than 60- and 90-day age groups of fish. The effects of cobalt on growth responses of three age groups (60, 90 and 120-day) of *Labeo rohita* were evaluated by Abbas and Javed (2016a). Feed intake, average wet weight and total length increments were found maximum in 120-day fish age group than 60 and 90-day age groups.

During the present investigation, it was found that all the three length groups of copper treated *C. marulius* and *M. seenghala* showed significantly lower growth in terms of an increase in average wet weights and total lengths than the control fish. These findings are in accordance with the results of Mickeniene *et al.* (2007). They conducted a trial to study the effects of sub-lethal copper concentrations on the growth performance of *Onchorhynchus mykiss* for a period of 20 days. Weight gain was observed maximum in control fish as  $133.10 \pm 2.37$  mg while it was minimum as  $106.10 \pm 3.76$  mg in copper treated fish. The present findings are also in conformity with the findings reported by Naz *et al.* (2008). They found that sub-lethal concentrations exposure of lead showed a statistically significant decrease in wet weights and lengths of major carps during 30-day growth trial. Hayat (2009) performed an experiment in which he treated major carps to sub-lethal metal concentrations and found less weight gain in stressed fish as compared to the control. During the present investigation, the condition factor was lower in control than copper

treated fish. Contradictory to present results, Giguere *et al.* (2004) found that the metals (copper, cadmium, and zinc) treated yellow perch (*Perca flavescens*) exhibited significantly lower condition factor than the control one. The feed intake of 50, 100, and 150 mm length groups of copper treated fish were significantly lower during present investigation as compared to the control fish groups. These results are similar to the findings of Witeska *et al.* (2010) who evaluated lower feed intake values in copper treated *Barbus barbus* in comparison to the fish without given metal stress, during growth trial of 14 days. Water-borne as well as dietary exposure of copper can reduce the feed intake and growth capability of *Clarias gariepinus* (Hoyle *et al.*, 2007). The present findings are also in conformity with Mohanty *et al.* (2009). They reported that as a result of sub-lethal copper stress, feed intake was reduced significantly ( $p < 0.05$ ) in *Cirrhina mrigala*. The feed conversion efficiency of control fish group was lower than copper treated fish during present investigation. Gharedaashi *et al.* (2013) found that  $\text{CuSO}_4$  treatment exerted negative impacts on feed conversion efficiency of *Rutilus frisii*. James *et al.* (2003) conducted an experiment in which they studied water-borne toxicity of copper on the growth performance of *Xiphophorus helleri* for 140 days. They observed a reduction in feed conversion efficiency as  $8.70 \pm 4.76\%$  in treated fish.

Species-specific variations in the growth potential as also observed during present investigation were also

recorded by Abbas and Javed (2016b). They conducted a 90-day trial to evaluate the impacts of sub-lethal concentrations of metals mixture (Pb+Zn+Mn) on the growth performance of *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Cirrhina mrigala*, *Labeo rohita* and *Catla catla*. Among all the fish species, *C. mrigala* exhibited significantly the highest average wet weight increment and specific growth rate. Ibrahim *et al.* (2008) found that metals (Pb, Cu, Zn and Cd) exposure exerted significantly varied impacts on the growth and condition factor of all the three fish species (*Tilapia zilli*, *Oreochromis niloticus* and *Sarotherodon galilaeus*). Statistically significant but least growth rate was recorded for *T. zilli* while the highest growth was found in *O. niloticus*. *S. galilaeus* exhibited the lowest condition factor value of 1.74 while *T. zilli* had the highest value as 1.94. Naz and Javed (2013) found species-specific variations in weight increments among five fish species when subjected to  $1/3^{\text{rd}}$  of  $\text{LC}_{50}$  exposure concentrations of Zn+Ni mixture for duration of 12 weeks. Increase in average wet weights among all the fish species followed the order: *Ctenopharyngodon idella* > *Labeo rohita* > *Hypophthalmichthys molitrix* > *Cirrhina mrigala* > *Catla catla*. The fish, *C. catla* exhibited significantly highest feed conversion efficiency, followed by *L. rohita*, *C. idella*, *C. mrigala* and *H. molitrix*.

It was concluded that the carnivorous fish species treated with sub-lethal copper concentrations

showed significantly reduced average wet weight and total length increments than the control fish that followed the order: *C. marulius* > *M. seenghala*. Among the three length groups of *C. marulius* and *M. seenghala*, 150mm fish length group exhibited significantly higher average wet weight and total length increments than 100mm and 50mm length groups of fish. The control fish group exhibited significantly lower condition factor as compared to the treated fish group. The condition factor computed for 50mm length group of both the fish species was significantly higher than 100mm and 150mm fish length groups. The copper treated *C. marulius* and *M. seenghala* exhibited significantly lower feed intake than control fish species. Among the three length groups of fish, the order of feed intake was 150 mm > 100 mm > 50mm. The overall means showed that feed conversion efficiency of the copper treated fish was significantly higher than the control fish. The FCE of three length groups of fish followed the order: 50 mm > 150 mm ≥ 100 mm. Among both the fish species, *C. marulius* exhibited significantly better FCE than *M. seenghala*. Overall, it was concluded from the present investigation that sub-lethal copper exposure caused significant reduction in the growth performance of carnivorous fish species. Also, the fish of young age became more susceptible to metallic ion (copper) stress ultimately showed lesser growth rate as compared to the old age fish. There were found species specific

growth differences among the both carnivorous fish species.

This study would be helpful for the environmentalists and policy makers so that they could devise strategies to limit the exposure of toxic metal i.e. copper in the natural aquatic ecosystems of Pakistan. Also, the results of present investigation would be beneficial for the fish farmers and consumers so that they become conscious to farm and consume metal exposed fish in order to avoid dreadful health consequences.

### Acknowledgement

The authors acknowledge the facilities and support offered by University of Agriculture, Faisalabad, Pakistan in the completion of this research work.

### References

- Abbas, S. and Javed, M., 2016a.** Growth performance of *Labeo rohita* under chronic dual exposure of water-borne and dietary cobalt. *Pakistan Journal of Zoology*, 48(1), 257-264.
- Abbas, S. and Javed, M., 2016b.** Growth performance of metals mixture stressed fish in earthen ponds. *Pakistan Journal of Zoology*, 48(3), 845-851.
- Abbas, S., Javed, M., Khan, H.A. and Rahman, K.U., 2018.** Toxicity and bioaccumulation of metals (Al and Co) in three economically important carnivorous fish species of Pakistan. *International Journal of Agriculture and Biology*, 20(5), 1123-1128.
- Ahmed, M.K., Baki, M.A., Kundu, G.K., Islam, M.S., Islam, M.M. and Hossain, M.M., 2016.** Human

- health risks from heavy metals in fish of Buriganga river, Bangladesh. *SpringerPlus*, 5(1), 1697.
- Ambreen, F. and Javed, M., 2016.** Effects of 30 day sub-lethal exposure of cadmium and lead mixture on DNA damage in fish. *The Journal of Animal and Plant Sciences*, 26(3), 674-679.
- Ameer, F., Javed, M., Hayat, S. and Abdullah, S., 2013.** Growth responses of *Catla catla* and *Labeo rohita* under mixed exposure of dietary and water-borne heavy metals viz. Cu, Cd and Zn. *Journal of Animal and Plant Sciences*, 23(5), 1297-1304.
- Ansari, T. M., Saeed, M. A., Raza, A., Naeem, M. and Salam, A., 2006.** Effect of body size on metal concentrations in wild *Puntius chola*. *Pakistan Journal of Analytical Chemistry*, 7(2), 116-119.
- APHA, 2012.** Standard methods for the examination of water and wastewater. 22<sup>nd</sup> ed. American Public Health Association. New York, 1360 P.
- Ashraf, A., Javed, M. and Abbas, S., 2018.** Investigation on the effects of zinc on gills peroxidase activity in the fish, *Labeo rohita*. *The Journal of Animal and Plant Sciences*, 28(3), 951-955.
- Atamanalp, M., Sisman, T., Geyikoglu, F. and Topal, A., 2008.** The histopathological effects of copper sulphate on rainbow trout liver (*Onchorynchus mykiss*). *Journal of Fisheries and Aquatic Science*, 3(5), 291-297.
- Atique, U., Iqbal, S., Khan, N., Qazi, B., Javeed, A., Anjum, K.M., Haider, M.S., Khan, T.A., Mahmood, S. and Sherzada, S., 2020.** Multivariate Assessment of Water Chemistry and Metals in a River Impacted by Tanning Industry. *Fresenius Environmental Bulletin*. 29, 3013–3025.
- Bae, D.Y., Atique, U., Yoon, J., Lim, B. and An, K.G., 2020.** Ecological Risk Assessment of Urban Streams Using Fish Biomarkers of DNA Damages and Physiological Responses. *Polish Journal of Environmental Studies*, 29, 1–10. DOI:10.15244/pjoes/104660
- Bhatti, M. Z., Rafiq, M. and Mian, A., 2013.** Kayrotype of sol (*Channa marulius*) from Indus river, Pakistan. *Journal of Animal and Plant Sciences*, 23(2), 475-479.
- Bogdanova, E.G., Ilyin, B.M. and Dragomilova, I.V., 2002.** Application of a comprehensive bias-correction model to precipitation measured at Russian North pole drifting stations. *Journal of Hydrometeorology*, 3, 700-713.
- DeForest, D.K. and Meyer, J.S., 2014.** Critical review: Toxicity of dietborne metals to aquatic organisms. *Critical Reviews in Environmental Science and Technology*, 45(11), 1176-1241.
- Doupe, R.G. and Lymbery, A.J., 2003.** Toward the genetic improvement of feed conversion efficiency in fish. *Journal of the World Aquaculture Society*, 34(3), 245-255.

- Gan, P., Ikeda, K., Irieda, H., Narusaka, M., Connell, R.J.O., Narusaka, Y., Takano, Y., Kubo, Y. and Shirasu, K., 2013.** Comparative genomic and transcriptomic analyses reveal the hemibiotrophic stage shift of *Colletotrichum* fungi. *New Phytologist*, 197(4), 1236-1249.
- Gharedaashi, E., Nekoubin, H., Imanpoor, M.R. and Taghizadeh, V., 2013.** Effect of copper sulfate on the survival and growth performance of Caspian Sea kutum, *Rutilus frisii* kutum. *Springer Plus*, 2, 498.
- Giguere, A., Campbell, P.G.C., Hare, L., McDonald, D.G. and Rasmussen, J.B., 2004.** Influence of lake chemistry and fish age on cadmium, copper, and zinc concentrations in various organs of indigenous yellow perch (*Perca flavescens*). *Canadian Journal of Fisheries and Aquatic Sciences*, 61(9), 1702-1716.
- Govind, P. and Madhuri, S., 2014.** Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Sciences*, 2(2), 17-23.
- Handeland, S.O., Imsland, A.K. and Stefansson, S.O., 2008.** The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. *Aquaculture*, 283, 36-42.
- Hayat, S., 2009.** Studies on the growth performance and meat quality of metal stressed fish reared under semi-intensive pond culture system. Ph.D. Thesis. Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan. pp. 1-195.
- Hoyle, I., Shaw, B.J. and Handy, R.D., 2007.** Dietary copper exposure in the African walking catfish, *Clarias gariepinus*: Transient osmoregulatory disturbances and oxidative stress. *Aquatic Toxicology*, 83(1), 62-72.
- Ibrahim, S.M., Shalloof, K.A.S. and Salama, H.M., 2008.** Effect of environmental conditions of Abu-Zabal lake on some biological, histological and quality aspects of fish. *Global Veterinaria*, 2(5), 257-270.
- Jadhav, J.P., Kalyani, D.C., Telke, A.A., Phugare, S.S. and Govindwar, S.P., 2010.** Evaluation of the efficacy of a bacterial consortium for the removal of color, reduction of heavy metals, and toxicity from textile dye effluent. *Bioresource Technology*, 101(1), 165-173.
- James, R., Sampath, K. and Edward, D.S., 2003.** Copper toxicity on growth and reproductive potential in an ornamental fish, *Xiphophorus helleri*. *Asian Fisheries Science*, 16, 317-326.
- Javed, M., 2015.** Assessment of genotoxic effects of metals in fish using comet and micronucleus assays. 1st Annual Report of PSF Project No. NSLP-285.
- Khanom, D.A., Nesa, A., Jewel, M.A.S., Haque, M.A., Paul, A.K., Iqbal, S., Atique, U. and Alam, L., 2020.** Muscular Tissue Bioaccumulation and Health Risk

- Assessment of Heavy Metals in Two Edible Fish Species (*Gudusia chapra* and *Eutropiichthys vacha*) in Padma River, Bangladesh. *Punjab University Journal of Zoology*, 35, 81–89.
- Mickeniene, L., Syvokiene, J. and Stasiunaite, P., 2007.** Effect of copper ions on growth and bacterial abundance in the intestinal tract of rainbow trout (*Oncorhynchus mykiss*) larvae. *Acta Zoologica Lituanica*, 17(1), 16-22.
- Mohanty, M., Adhikari, S., Mohanty, P. and Sarangi, N., 2009.** Role of waterborne copper on survival, growth and feed Intake of Indian major carp, *Cirrhinus mrigala* Hamilton. *Bulletin of Environmental Contamination and Toxicology*, 82(5), 559-563.
- Moon, W.K., Atique, U. and An, K.G., 2020.** Ecological risk assessments and eco-toxicity analyses using chemical, biological, physiological responses, DNA damages and gene-level biomarkers in Zebrafish (*Danio rerio*) in an urban stream. *Chemosphere*, 239, 124754.
- Moorthikumar, K. and Muthulingam, M., 2010.** Shifts in protein metabolism in liver, kidney and brain of Indian major carp, *Labeo rohita* (Hamilton) under heavy metal, nickel chloride stress. *International Journal of Current Research*, 7, 14-17.
- Naz, S., Javed, M., Hayat, S., Abdullah, S., Bilal, M. and Shaukat, T., 2008.** Long term effects of lead (Pb) toxicity on the growth performance, nitrogen conversion ratio and yield of major carps. *Pakistan Journal of Agricultural Sciences*, 45(3), 53-58.
- Naz, S. and Javed, M., 2013.** Growth responses of fish during chronic exposure of metal mixture under laboratory conditions. *Pakistan Veterinary Journal*, 33(3), 354-357.
- Parasuraman, S., 2011.** Toxicological screening. *Journal of Pharmacology and Pharmacotherapeutics*, 2(2), 74-79.
- Prasath, P.M.D. and Arivoli, S., 2008.** Biochemical study of freshwater fish *Catla catla* with reference to mercury chloride. *Iranian Journal of Environmental Health, Science and Engineering*, 5(2), 109-116.
- Rafique, M. and Khan, N.U.H., 2012.** Distribution and status of significant freshwater fishes of Pakistan. *Records Zoological Survey of Pakistan*, 21, 90-95.
- Rauf, A., Javed, M. and Ubaidullah, M., 2009.** Heavy metal levels in three major carps (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) from the river Ravi, Pakistan. *Pakistan Veterinary Journal*, 29(1), 24-26.
- Richter, T.J., 2007.** Development and evaluation of standard weight equations for bridgelip suckers and largescale suckers. *North American Journal of Fisheries Management*, 27(3), 936-939.
- Saeed, F., Iqbal, K.J., Atique, U., Javid, A., Khan, N., Iqbal, S., Majeed, H., Azmat, H., Khan, B.Y.A., Baboo, I., Shahid, M.T. and Afzal, G., 2020.** Toxic trace

- metals assessment in selected organs of edible fish species, sediment and water in Head Punjnad, Punjab, Pakistan. *Punjab University Journal of Zoology*, 35, 43–50.
- Skouras, A., Broeg, K., Dizer, H., Westernhagen, H.V., Hansen, P.D. and Steinhagen, D., 2003.** The use of innate immune responses as biomarkers in a programme of integrated biological effects monitoring on flounder (*Platichthys flesus*) from the southern North sea. *Helgoland Marine Research*, 57(3-4), 190-198.
- Subathra, S. and Karuppasamy, R., 2007.** Toxic effects of copper on bioenergetics and growth rates in fingerlings and adult age of the fish, *Mystus vittatus* (Bloch, 1794). *Journal of Fisheries and Aquatic Science*, 2(4), 285-293.
- Sundarraaj, S., Thangam, R., Sujitha, M.V., Vimala, K. and Kannan, S., 2014.** Ligand-conjugated mesoporous silica nanorattles based on enzyme targeted prodrug delivery system for effective lung cancer therapy. *Toxicology and Applied Pharmacology*, 275(3), 232-243.
- Vutukuru, S.S., 2005.** Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. *International Journal of Environmental Research and Public Health*, 2(3-4), 456-462.
- Wepener, V., Van Vuren, J.H.J. and Du Preez, H.H., 2001.** Uptake and distribution of a copper, iron and zinc mixture in gill, liver and plasma of a freshwater teleost, *Tilapia sparrmanii*. *Water SA*, 27(1), 99-108.
- Witeska, M., Bilska, K. and Sarnowski, P., 2010.** Effects of copper and cadmium on growth and yolk utilization in barbel (*Barbus barbus* L.) larvae. *Polish Journal of Environmental Studies*, 19(1), 227-230.
- Yang, H. and Rose, N.L., 2003.** Distribution of mercury in six lake sediment cores across the UK. *The Science of the Total Environment*, 304(1-3), 391-404.