ACTA ABP BIOCHIMICA POLONICA

Vol. 70, No 4/2023
943–949
https://doi.org/10.18388/abp.2020_6864

Regular paper

# Relative expression levels of growth hormone gene and growth rate in Indian major carp species

Shahid Sherzada[1], Muhammad Nauman Sharif[2], Qurban Ali[3], Saeed Akram Khan[1], Tawaf Ali Shah[4], Mohamed A. M. El-Tabakh[5], Tariq Aziz[6], Ghulam Nabi[7], Metab Alharbi[8], Thamer H. Albekairi[8] and Abdullah F Alasmari[8]

[1]Department of Zoology, Government College University Lahore, Pakistan; [2]Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan; [3]Department of Plant Breeding and Genetics, University of the Punjab, Lahore, Pakistan; [4]Department of Biotechnology, University of Okara, Punjab, Pakistan; [5]Zoology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt; [6]Laboratory of Animal Health, Food Hygiene and Quality, Department of Agriculture, University of Ioannina, 47132 Arta, Greece; [7]Institute of Nature Conservation Polish Academy of Sciences Krakow Poland; [8]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

**The phenomenon of growth is a leading factor for aquaculture success. The uneven growth of major Indian carps (*Labeo rohita, Catla catla,* and *Cirrhinus mrigala*) is a serious issue in fish culture from an economic point of view. The growth hormone (GH) gene is crucial for selection in commercially cultivated fish species for better growth and production. Indian major carp (*L. rohita, C. catla,* and *C. mrigala*) are commonly cultured in Pakistan. The *GH* expression was examined using qPCR to understand growth in fish species better. Muscle tissue samples (*n*=480) from 160 individuals of the same age were collected from three species (*L. rohita, C. catla,* and *C. mrigala*). Individuals were divided into two groups (high-weight and low-weight groups), cultured under normal conditions. The housekeeping gene β-actin validated *GH* expression in fast and slow-growing fishes from the same species. Results showed that *GH* expression varies across species and fish specimens that overweight their counterpart feature have higher *GH* expression. A selection for overweight fish in the aquaculture breeding systems is preferable as those fish could inherit their genomics to the future cohort, enhancing production, and commercial profit for farmers. Comprehensive research about different growth genes and the environmental aspects that influence fish growth is mandatory. No work has been reported regarding the growth gene analysis of fish from Pakistan. This report was Pakistan's first and baseline study regarding growth analysis of main culturable fish species at the molecular level.**

**Keywords**: *Labeo rohita, Cirrhinus mrigala, Catla catla*, Growth hormone, Beta-actin gene, Q-PCR

**Received**: 02 June, 2023; revised: 20 September, 2023; accepted: 02 November, 2023; available on-line: 14 November, 2023

e-mail: shahid.sherzada@gcu.edu.pk (SS); iwockd@gmail.com (TA)
**Acknowledgments**: Authors are thankful to the Researchers Supporting Project number (RSPD2023R568), King Saud University, Riyadh, Saudi Arabia.
**Abbreviations**: cDNA, complementary DNA; GH, growth hormone; RT-PCR, reverse transcriptase Polymerase Chain Reaction

## INTRODUCTION

Pakistan is blessed with fish fauna and water resources, but only nine species (7 warm and 2 cold water) are commercially cultured. Moreover, only carp culture is carried out through extensive farming depending on natural water resources. Among polyculture, carp poly-culture systems primarily consisting of major carps (*Labeo rohita, Catla catla, and Cirrhinus mrigala*) are more prevalent due to their high growth rate and ability to adapt in captive conditions (FAO, 2016; Wasim, 2007). Aquaculture success is mainly dependent on the maximum growth and production of fish. Vertebrate growth is regulated by several genes and various morphological characters (Dunham, 2011). The growth expression is correlated with a screening of candidate genes involved in the growth and selection of fish species. Higher fish growth is essential for boosting the aquaculture industry (Khan *et al.,* 2023; Canestro *et al.,* 2013). The role of the *GH* is extensively studied in many fishes like Mandarin fish (*Siniperca chuatsi*; Yi *et al.,* 2015), Nile tilapia (*Tilapia nilotica*; Tanamati *et al.,* 2015), Tench (*Tinca tinca*; Kocour *et al.,* 2011), Ganges Jewfish *(Nibea coibor*; Zhang *et al.,* 2009), and Mandarin fish (*Siniperca chuatsi*; Tian *et al.,* 2014). In recent years, marker-assisted growth selection has been employed in the aquaculture and livestock sector for better performance (Ling *et al.,* 2023; Biswas *et al.,* 2003; Pal *et al.,* 2004; De Faria *et al.,* 2006).

Fish growth is a key feature for many fish biologists regarding fish production and the selective breeding program of many economically significant fish species (Gjedrem *et al.,* 2012; Hulata, 2001). Growth is a quantitative trait controlled by many genes (Mullis, 2005) and environmental factors (Mackay, 2001; Moriyama *et al.,* 2000). The *GH*, growth hormone receptor (*GHR)*, insulin-like growth factors *(IGF)*, and myostatin (*MSTN)* genes are observed in tamed animals and aquaculture species. Among them, *GH* is the main regulator of postnatal development. Moreover, it is also associated with strengthening anabolic functions such as protein interaction, cell growth, and muscle development (Reinecke *et al.,* 2005). In addition to hormone-flagging procedures, it participates in other pathways that make its physiology challenging to understand (Vairamani *et al.,* 2017). The primary genes that control fish growth have been studied using various experimental methods. Research on such fish ranges from those whose growth has been retarded through starvation during gametogenesis to those whose growth has been accelerated through refeeding after starvation, GH-transgenesis, or domestication. These studies also discovered a complicated association among factors that control growth and other biological processes. Moreover, it is also

**Table 1. Feeding and water quality monitoring data during tissue sampling.**

| Seed Rearing Stage | | | | | Production Stage |
|---|---|---|---|---|---|
| Total Age: 19 Months | Jun–Aug | Aug–Nov | Dec–Jan | February | March–October |
| Feed (CP level %) | Rice polish CP 14 | Supreme feed CP 24 | Partial feeding: Low temperature CP 26 | Partial feeding: Low temperature CP 26 | Supreme feed CP 31 |
| *DO (ppm) | 5.5–6.5 | 5.5–6.5 | 6.0–7.0 | 6.5–8.5 | 5.2–7.5 |
| Temperature (°C) | 29–32 | 27–29.5 | 19–23 | 20–25 | 24–31 |
| pH | 6.8–8.7 | 7.9–9.2 | 8.6–9.5 | 8.2–9.4 | 7.4–8.8 |
| Salinity(ppt) | <0.09 | <0.09 | <0.09 | <0.09 | <0.09 |
| *TDS (ppm) | 1900–2300 | 2100–2500 | 2300–2590 | 2295–2476 | 2340–2700 |
| Ammonia (ppm) | 0.012–0.024 | 0.018–0.03 | 0.03–0.041 | 0.025–0.039 | 0.028–0.065 |

*DO, dissolved oxygen; *TDS, total dissolved solid

revealed that many somatotropic genes such as *GH*, *GHR*, and insulin-like growth factors *IGFs* are more expressed in fast-growing fishes, i.e., rainbow trout (*Oncorynchus mykiss*) (Devlin *et al.,* 2013) and regulate muscle growth (Sheridan & Hagemeister, 2010).

GH is secreted by somatotropic cells and comprises a single-chain polypeptide of about 22kDa. It plays a vital role in metabolic processes, i.e., reproductive and immune systems and seawater adaptability. Moreover, it is also involved in problems related to appetite and food conversion (Rajesh & Majumdar, 2007; Canosa *et al.,* 2007). Previously DNA and cDNA sequences of the *GH* have been well studied in various economically important fish species like *Cyprinus carpio* (Chiou *et al.,* 1990), *Protopterus annectens* (May *et al.,* 1999), *Cynoglossus semilaevis* (Ma *et al.,* 2012), and *Pelteobagrus fulvidraco* (Li *et al.,* 2017). The total length of the *GH* was almost 3-4kb and its structures differed in different fishes. The *GH* genome in some fishes consists of 5 exons and 4 introns; in other fish species, it was 6 exons and 5 introns (Chiou *et al.,* 1990; Li *et al.,* 2017). The current study was undertaken to determine the differential expression levels of the *GH* in fast and slow-growing fish species of Indian major carps (*Labeo rohita*, *Catla catla,* and *Cirrhinus mrigala*), which are commonly cultured in Pakistan.

## MATERIALS AND METHODS

### Sample Collection for Growth Expression Analysis

A total of 480 fish individuals (*Labeo rohita*/*n*=160, *Cirrhinus mrigala*/*n*=160, and *Catla catla*/*n*=160) of similar age were collected from the Umer Fish Farm, located at District Rajanpur, Pakistan. The capturing and experiments on fish species were conducted following relevant institutional, national, and international guidelines and legislation. Out of 160 muscle tissue samples (Skeletal Muscle, 5 g) from each fish species, 80 samples were captured from extremely high-weight fishes (≥1.3; ≥2 and ≥0.9 Kg) and 80 from extremely low-weight fishes (≤0.5; ≤1 and ≤0.5 kg) from *L. rohita, C. catla,* and *C. mrigala,* respectively. Fish samples were collected in a centrifuge tube (1.5 ml), containing RNA later solution. Samples were stored immediately in liquid nitrogen and then at –80°C for later analysis. During sampling, feeding and water quality data were continuously collected (Table 1).

### Total RNA isolation and cDNA synthesis

Total RNA isolation from the muscle tissue sample was carried out using TRIzol reagent (Life Technologies cat no. 15596026). Samples were ground in liquid nitrogen using a pre-chilled mortar and pestle. The homogenate of muscle tissue samples was made in a 2.0 ml microcentrifuge tube with 1.5 mL of TRIzol® reagent and incubated for 5 minutes at room temperature. After shaking strongly for 30–40 seconds, 200 µl of chloroform was added per 1 ml of TRIzol® reagent and the content was mixed vigorously for 15 seconds and incubated at room temperature for 10 minutes followed by centrifugation for 5 minutes at 13000 rpm (at 4°C). As a result of centrifugation, three distinctive layers were formed consisting of the upper colorless, middle whitish layer and bottom dark layer. The upper clear phase containing nucleic acid was transferred to another nuclease-free 2.0 ml Eppendorf tube and 750 µl of pre-chilled isopropanol was added to precipitate the RNA and incubated for 10 minutes at room temperature followed by centrifugation. To clean the RNA pellet, 750 µl of 75% ethanol was used and centrifuged again at 13000 rpm (at 4°C) for 15 minutes (2×) by discarding the upper content. The RNA pellet was air-dried by incubating at room temperature for 10 minutes. The air-dried RNA samples were resuspended in 30 µl DEPC water and immediately stored at -70°C. The quality and quantity of the isolated RNA were determined by using Spectrophotometer ND 1000 NanoDrop®. and the Size of RNA was determined by 1.5 % gel electrophoresis (Fig. 1).

### Synthesis of complementary DNA (cDNA)

The cDNA was synthesized using the Revert Aid First-Strand cDNA synthesis kit (Thermo Fisher Sci-

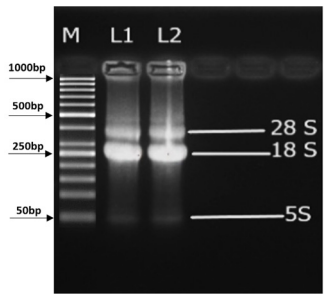


**Figure 1. Confirmation of RNA by gel electrophoresis (1.5%)**

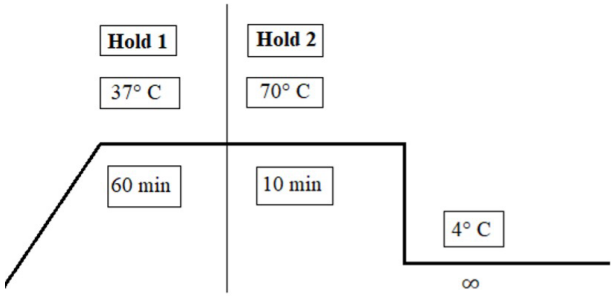


**Figure 2. Thermal conditions for cDNA synthesis**

entific, cat no. 1622). 1 µg of total RNA template isolated from each fish sample was used to synthesize cDNA. The reaction mixture for cDNA synthesis contained 10 pm of random hexamer primers, 1.2 µl of RNA (1 µg) and nuclease-free DEPC treated water was used to make a final volume of 11 µl. PCR tube containing the cDNA mixture was centrifuged for 5–10 seconds. The reaction mixture was incubated at 70°C for 5 minutes and tubes were immediately placed on ice. After that 4.0 µl of 5x reaction buffer, 1.0 µl (20 units) of ribonuclease inhibitor, and 2.0 µl of dNTPs mixture (40 mM) were supplemented. The reaction mixture was incubated for 5 minutes at 37°C and 2.0 µl (40 units) of reverse transcriptase (MMuLV) was supplemented. The final reaction mixture of 20 µl was finally incubated at 37°C for 60 minutes. The cDNA reaction mixture was additionally incubated at 70°C for 10 minutes to break the reaction. The obtained cDNA product was immediately placed on ice. The thermal profile of the reaction is shown below.

**Table 2. RT-PCR primers selection**

| Gene | Primers | Sequence of primers | Product size |
|---|---|---|---|
| GH | *GH*–F | ATAACGACTCCCTGCCACTG | 125 bp |
| | *GH*–R | CCTTGTGCATGTCCTTCTTG | |
| β-actin | β-actin-F | ACCCACACTGTGCCCATCTACG | 146 bp |
| | *β-actin*-R | ATTTCCCTCTCGGCTGTGGTGG | |

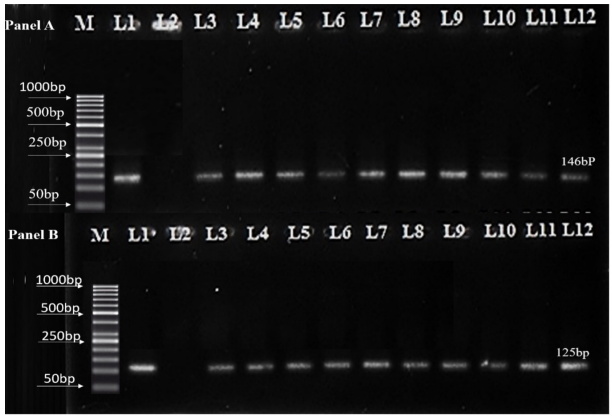


**Figure 3. Primer's optimization for RT-PCR.**
Panel (**A**) Beta-actin amplification (fragments=146bp: L3-L12); M=Marker (50bp); L1=Positive control; L2=Negative control. Panel (**B**) Growth hormone gene (GH) amplification (fragments=125bp: L3-L12); M=Marker (50bp); L1=Positive control; L2=Negative Control.

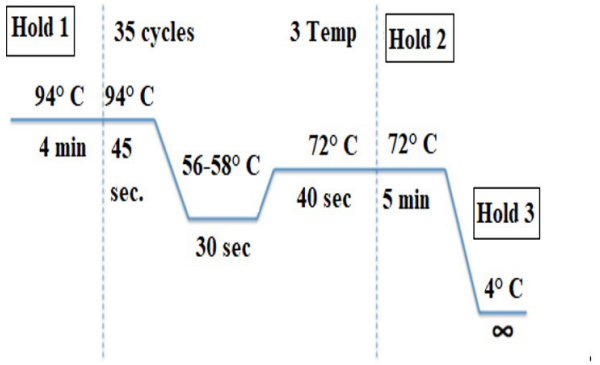


**Figure 4. RT-PCR Amplification thermal conditions to amplify targeted genes**

### Primer Designing and Optimization

Primer Express 3.0 software was used to design primers for the *GH* and housekeeping genes (*β-actin).* The *β-actin* was selected as an internal control for precise validation of qPCR (Table 2). Both primers (*GH* and *β-actin)* were optimized using certain qPCR conditions. The results of the optimized protocol were assessed by the gel documentation system (Fig. 3).

### Amplification of targeted genes by reverse transcriptase Polymerase Chain Reaction (RT-PCR)

Real-time PCR was used to quantify differential *GH* expression of fishes with the highest and lowest weight under uniform pond conditions. The RT-PCR process was accomplished using PikoReal™ Q-PCR to quantify selected samples. Amplification of the targeted genes was carried out using gene-specific primers (Table 2) and DreamTaq Green PCR Master mix 2x (Thermo Fisher Scientific, cat no. K1081). The PCR reaction contained 10 µ-L of DreamTaq Green PCR master mix, 0.8 µ-L template cDNA and 0.4 µ-L of both forward and reverse primers (10 µ-mol) added to PCR tube (0.2 mL) and nuclease-free water to a final volume of 20 µ L. The PCR thermal conditions were; preliminary incubation for 4 minutes at 94°C, 30 cycles of amplifications with denaturation at 94°C for 45 seconds, primer binding at 56–58°C for 30 seconds and amplification at 72°C for 40 seconds and then ending extension for 5 minutes at 72°C (Fig. 4). The 2–ΔΔCT mode was selected to assess *GH* expression (Livak & Schmittgen, 2001).

### Data analysis

Data were coded and entered using the statistical package SPSS (IBM SPSS Statistics V.22). The data were checked for satisfying parametric test assumptions, and continuous variables were tested for normality using Shapiro-Wilk and Kolmogorov-Smirnov tests. Data were presented as mean and standard deviation. A student *t*-test was conducted for experimental groups, while a Tukey pairwise comparison was conducted in MiniTab V14 for post-hoc analysis. The *P*-value was considered significant at<0.05. Data was visualized when possible, using R studio V 2022.02.4.

## RESULTS

In this study, we observed significant differences in body weights between high-weight and low-weight groups of similar ages in all three fish species (Fig. 5).

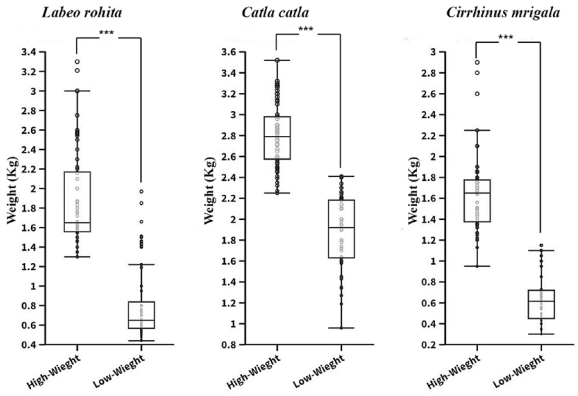


**Figure 5. Box and whisker plot of observed weight for investigated fish species.**
Data undergoes an independent sample t-test, after being evaluated for normality. NS=non-significant; *<0.05; **<0.01: ***<0.001.

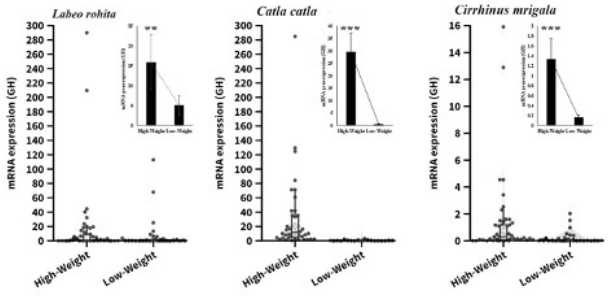


**Figure 6. Comparative analysis of differential growth hormone gene expression (*GH*) among three fish species having a High-Weight and Low-Weight group.**
Data undergoes the Mann-Whitney U test, after being evaluated for normality. NS=non-significant; *<0.05; **<0.01: ***<0.001

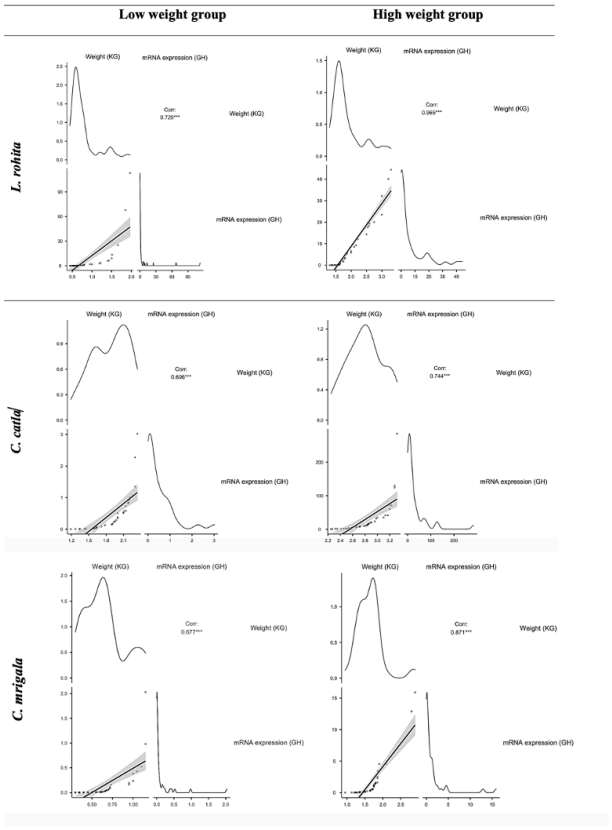


**Figure 7. Correlation coefficient between observed fish weight in low and high weight groups and the gene expression values of GH hormone**
NS=non-significant; *<0.05; **<0.01: ***<0.001

## Differential GH Analysis

### *Labeo rohita*

Average values of *GH* expression and body weight of selected fish samples were utilized to determine the relationship between *GH* expression and body weight. Larger body weight fishes (1.88±0.50 kg) have significantly higher *GH* expression relative to lower body weight fishes (0.83±0.38 kg) (Fig. 6).

### *Catla catla*

Similar to *Labeo rohita*, individuals having high body weight (80±0.30 Kg) had significantly higher *GH* expression than lower body weight individuals (Fig. 6).

### *Cirrhinus mrigala*

Like the other two fish species, *Cirrhinus mrigala* expressed *GH* significantly higher in higher body weight fishes (1.65±0.36 Kg) as compared to lower body weight fishes (0.65±0.25; Fig. 6).

We found a very surprising correlation between *GH* expression and the body weight of selected fish species (Fig. 7). The analysis revealed a statistically significant positive correlation between body weight and gene expression for both groups of low and high body weight fish. The strength of association was greater in the high-weight groups compared to the low-weight groups for all three species. For *L. rohita*, the correlation coefficient was 0.965 and 0.729 for the high and low-weight groups, respectively. Similarly, for *C. catla* the correlation coefficients were 0.744 and 0.696, and for *C. mrigala* they were 0.871 and 0.677 for the high and low-weight groups, respectively. In summary, the degree of correlation between body size and gene expression intensified as body weight increased for all species.

The analysis revealed a statistically significant positive correlation between body weight and gene expression for both groups of fish with low and high body weight. The strength of association was greater in the high-weight groups compared to the low-weight groups for all three species. For *L. rohita*, the correlation coefficient was 0.965 and 0.729 for the high and low weight groups, respectively. Similarly, for *C. catla* the correlation coefficients were 0.744 and 0.696, and for *C. mrigala* they were 0.871 and 0.677 for the high and low weight groups, respectively. In summary, the degree of correlation between body size and gene expression intensified as body weight increased for all species.

## DISCUSSION

Growth in vertebrates is mainly controlled by GH, a vital gene influencing growth physiology (Quik *et al.,* 2010; Clayton *et al.,* 2010). Indian major carp species (*Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*) are widely cultured in Asia. *GH* might directly or indirectly affect the growth mechanism of these economically important fish species. We therefore investigated the connection of *GH* expression with the growth characteristics of carp species. In recent years, *GH* association with growth traits

has been studied well in domestic animals (An *et al.,* 2011; Sugita *et al.,* 2014) and poultry (Xu *et al.,* 2007; Wu *et al.,* 2012). The GH is the chief controller of IGF-I production in fry, fingerling, and adult fishes. Studies reported that injecting GH boosts the expression of IGF-I in salmon, (Duguay *et al*., 1994), rainbow trout (Shamblott *et al.,* 1995), and gilthead sea bream (Duguay *et al.,* 1996), zebrafish (Maures *et al.,* 2002). The *IGF-I* mRNA levels in plasma or serum and its expression in tissues also confirmed its dependency on GH as observed in goldfish (Marchant & Moroz, 1993), salmonids (Moriyama *et al.,* 1995) and tilapia (Kajimura *et al.,* 2001).

We reported that *GH* expression varies in high and low-body-weight fish species of Indian major carp. The variable expression of growth-related genes has also been observed in fish and other animals in previous scientific studies (Carnevali *et al.,* 2006; Filby & Tyler, 2007; Opazo *et al.,* 2017). In fish, somatic growth is accelerated at the juvenile stage while it slows down during the adult stage as fish utilizes its energy for gonadal development and sexual maturation (Le Gac *et al.,* 1993; Chen *et al.,* 2018). In the present study, fish with the same age class and different body weights exhibit high *GH* expression as fish weight increases. Differences in recorded body weight of fish within the same age group could rely on stress factors, that can minimise fish growth and production. This stress may be caused due to internal factors (stocking density, fish species' competition for space and food) or external factors (low water quality) (Karakatsouli *et al.,* 2008). If the stress factor is negated, then the role of the gene can be observed and explained well in experimental fishes, which enlightens the present study findings. Considering this scenario, present cultured fishes undergo uniform culturing conditions by following good feeding practices and healthy water quality management so that possible genetic effects on fish growth can be assessed effectively. The differential expression level of *GH* in three fish species defined that fish growth rates are different concerning *GH* content in muscle tissue. Similar observations were recorded in red spotted grouper (*Epinephelus akaara*) regarding expression analysis of growth genes like *GH*, *GHR*, and *IGF1* from fish tissue (Mun *et al.,* 2019).

A direct relationship was noticed between body weight and *GH* expression in *L. rohita*, which depicts that expression of the *GH* increases or decreases with the gradual increase or decrease in body weight of fish samples. *GH*-mRNA expression was found higher in a high-weight group while lower in low-weight populations, similar to those mentioned for crucian carp *C. auratus* (Zhong *et al.,* 2012); European sea bass (*Dicentrarchus labrax*), which had a significantly higher *GH*-mRNA expression in fast-growing as compared to slow-growing fish samples (Carnevali *et al.,* 2006). However, such differential *GH*-mRNA expression level was not obvious in zebrafish, *Daniorerio* (Opazo *et al.,* 2017). Similar to *L. rohita,* we found the same findings for *C. catla*. Results of *GH* expression concerning the body weight of *C. catla* fish samples were very interesting as we found the same high per unit expression of *GH* with respect to a gradual increase in weight in higher body weight fishes. Such kind of growth pattern may reflect some ongoing interconnected morphological as well as physiological changes inside the body of *C. catla*. In contrast, a low level of *GH* expression was reported in high-body weight fish of Nile Tilapia (*Oreochromis niloticus*), which may be compensated by expression in some other tissues of the fish body (Aboukila *et al.,* 2021). The low level of *GH* expression may depict its low synthesis from the pituitary due to the influence of endocrine and environmental aspects correlated with the body physiology, suggesting that *GH* expression in fish is a very complicated phenomenon (Tymchuk *et al.,* 2009).

A direct correlation was also identified between body weight and *GH* expression in collected *C. mrigala*. Such results indicated a higher chance of reciprocal effect of *GH* expression in low-body-weight fish than in the high-body-weight group. A previous study on *GH* expression analysis of red-spotted grouper revealed that *GH*-mRNA expression was slightly higher in higher body weight samples than in moderate and slow-growing samples (Mun *et al.,* 2019). Likewise, in *C. mrigala GH* expression results, a marine water fish named gilthead sea bream (*Sparus aurata*) possessed similar influential growth patterns of *GH* on high and low-weight fish tissues (Perez-Sanchez *et al.,* 1994). Comparing *GH* expression among high and low body weight Indian major carp species revealed that *GH* expression varies from species to species in these fishes, however, it follows a similar trend with increasing or decreasing body weight. Certain other vital growth genes and various ambient environmental factors important for fish's body development and growth may cause current variation in expression results. The intricate phenomenon of the endocrine system in fishes reveals that the growth pattern with respect to hormonal state is very complex. This pattern gradually fluctuates among fresh and marine water fish species. More research in this area is necessary to properly understand growth governing endocrinology in fish.

## CONCLUSION

Our research focused on *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala*, which are major Indian carp species commonly cultured in Pakistan. The uneven growth of these species is a significant challenge in aquaculture, impacting economic outcomes. The growth hormone (GH) gene is pivotal in selecting fish with superior growth and production potential in aquaculture. By validating GH expression using the housekeeping gene β-actin, we discovered that GH expression varies not only among species but also within the same species based on weight, with better-weight fish exhibiting higher GH expression levels. The significance of our findings lies in their potential application within the aquaculture sector in Pakistan. Selecting and breeding better-weight fish, which demonstrate higher GH expression can lead to improved production outcomes and increased profitability for farmers. Furthermore, this study represents the first molecular-level analysis of growth genes in fish species from Pakistan, making it a baseline study for future research in this region.

### Declarations

**Ethical Approval**. It has been confirmed that the experimental sample, including the collection of fish blood samples, complied with relevant institutional, national, and international guidelines and legislation with appropriate approval from the Ethical Review Committee of the Department of Zoology, Government College University Lahore, Pakistan (Approval ID: Fish2570). It has been confirmed that the study was reported following the Guidelines for the Use of Fishes in Research (AFS 2014).

**Competing interests.** There are no competing interests regarding the publication of this manuscript.

**Funding.** Currently, no funding is available.

**Availability of data and materials.** All data generated or analyzed during the study are included in the manuscript.

## REFERENCES


Aboukila RS, Hemeda SAE, El Nahas AF, Abd El Naby WSH (2021) Molecular characterization of GHR1 gene and expression analysis of some growth-related genes in *Oreochromis niloticus*. *Adv Anim Vet Sci* **9**: 1025–1033. https://doi.org/10.17582/journal.aavs/2021/9.7.1025.1033

An X, Wang L, Hou J, Li G, Song Y, Wang J, Yang M, Cui Y, Cao B (2011) Novel polymorphisms of goat growth hormone and growth hormone receptor genes and their effects on growth traits. *Mol Biol Rep* **38**: 4037–4043. https://doi.org/10.1007/s11033-010-0522-3

Bhatta S, Iwai T, Miura T, Higuchi M, Maugars G, Miura C (2013) Differences between male and female growth and sexual maturation in tilapia (*Oreochromis mossambicus*). *Kathm Univ J Sci Eng Techn* **8**: 57–65. https://doi.org/10.3126/kuset.v8i2.7326

Biswas TK, Bhattacharya TK, Narayan AD, Badola S, Kumar P, Sharma A (2003) Growth hormone gene polymorphism and its effect on birth weight in cattle and buffalo. *Anim Breed Genet* **16**: 494–497. httpss://doi.org/10.5713/ajas.2003.494

Canestro C, Albalat R, Irimia M, Garcia-Fernandez J (2013) Impact of gene gains, losses and duplication modes on the origin and diversification of vertebrates. *Semin Cell Dev Biol* **24**: 83–94. https://doi.org/10.1016/j.semcdb.2012.12.008

Canosa LF, Chang JP, Peter RE (2007) Neuroendocrine control of growth hormone in fish. *Gen Comp Endocrinol* **151**: 1–26. https://doi.org/10.1016/j.ygcen.2006.12.010

Carnevali O, Vivo LD, Sulpizio R, Gioacchini G, Olivotto I, Silvi S, Cresci A (2006) Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*) with particular attention to IGF-1, myostatin and cortisol gene expression. *Aquac* **258**: 430–438. https://doi.org/10.1016/j.aquaculture.2006.04.025

Chen JI, Cao M, Zhang A, Shi M, Tao B, Li Y, Wang Y, Zhu Z, Trudeau VL, Hu W (2018) Growth hormone overexpression disrupts reproductive status through actions on leptin. *Front Endocrinol* **9**: 131. https://doi.org/10.3389/fendo.2018.00131

Chiou CS, Chen HT, Chang WC (1990) The complete nucleotide sequence of the growth-hormone gene from the common carp (*Cyprinus carpio*). *Biochim Biophys Acta* **1087**: 91–94. https://doi.org/10.1016/0167-4781(90)90126-m

Clayton PE, Indraneel B, Philip GM, Andrew GR (2010) Growth hormone, the insulin-like growth factor axis, insulin and cancer risk. *Nat Rev Endocrinol* **7**: 11–24. https://doi.org/10.1038/nrendo.2010.171

Contreras-Sanchez WM, Schreck CB, Fitzpatrick MS, Pereira CB (1998) Effects of Stress on the Reproductive Performance of Rainbow Trout (*Oncorhynchus mykiss*). *Biol Reprod* **58**: 439–447. httpss://doi.org/10.1095/biolreprod58.2.439

De-Santis C, Jerry DR (2007) Candidate growth genes in finfish—Where should we be looking? *Aquac* **272**: 22–38. https://doi.org/10.1016/j.aquaculture.2007.08.036

De-Faria DA, Guimaraes SEF, Lopes PS, Pires AV, Paiva SR, Sollero BP, Wenceslau AA (2006) Association between G316A growth hormone polymorphism and economic traits in pigs. *Genet Mol Biol* **29**: 634–640. https://doi.org/10.1590/s1415-47572006000400010

Devlin RH, Sakhrani D, White S, Overturf K (2013) Effects of domestication and growth hormone transgenesis on mRNA profiles in rainbow trout (*Oncorhynchus mykiss*). *J Anim Sci* **91**: 5247–5258. https://doi.org/10.2527/jas.2013-6612

Duguay SJ, Lai-Zhang J, Steiner DF, Funkenstein B, Chan SJ (1996) Developmental and tissue-regulated expression of IGF-I and IGF-II mRNAs in *Sparus aurata*. *J Mol Endocrinol* **16**: 123–132. https://doi.org/10.1677/jme.0.0160123

Duguay SJ, Swanson P, Dickhoff WW (1994) Differential expression and hormonal regulation of alternatively spliced IGF-I mRNA transcripts in salmon. *J Mol Endocrinol* **12**: 25–37. https://doi.org/10.1677/jme.0.0120025

Dunham RA (2011) Aquaculture and fisheries biotechnology: Genetic approaches. CABI Publishing, *Wallingford,* UK, pp 495. https://doi.org/10.1079/9781845936518.0000

FAO (2016) IFAD. The State of Food Insecurity in the World 2015. Meeting the 2015 international hunger targets: taking stock of uneven progress. 2015. *Rome*. https://doi.org/10.3945/an.115.009936

Filby AL, Tyler CR (2007) Cloning and characterization of cDNAs for hormones and/or receptors of growth hormone, insulin-like growth factor-I, thyroid hormone, and corticosteroid and the gender-, tissue-, and developmental specific expression of their mRNA transcripts in fathead minnow (*Pimephales promelas*). *Gen Comp Endocrinol* **150**: 151–163. https://doi.org/10.1016/*j.ygcen*.2006.07.014

Gjedrem T, Robinson N, Rye M (2012) The importance of selective breeding in aquaculture to meet future demands for animal protein: a review. *Aquac* **350**: 117–129. https://doi.org/10.1016/*j.aquaculture*.2012.04.008

Hulata G (2001) Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. *Genetica* **111**: 155–173. https://doi.org/10.1023/a:1013776931796

Kajimura S, Uchida K, Yada T, Riley LG, Byatt JC, Collier RJ, Aida K, Hirano T, Grau EG (2001) Stimulation of insulin-like growth factor-I production by recombinant bovine growth hormone in Mozambique tilapia, *Oreochromis mossambicus*. *Fish Physiol Biochem* **25**: 221–230. https://doi.org/10.1023/a:1022268811599

Karakatsouli N, Papoutsoglou SE, Pizzonia G, Tsatsos G, Tsopelakos A, Chadio S, Kalogiannis D, Polissidis A, Papadopoulou-Daifoti Z (2008) Effects of light spectrum on growth and physiological status of gilthead seabream *Sparus aurata* and rainbow trout *Oncorhynchus mykiss* reared under recirculating system conditions. *Aquac Eng* **36**: 302–309. https://doi.org/10.1016/j. aquaeng.2007.01.005

Khan BN, Ullah H, Ashfaq Y, Hussain N, Atique U, Aziz T, Alharbi M, Albekairi TH, Alasmari AF (2023) Elucidating the effects of heavy metals contamination on vital organ of fish and migratory birds found at fresh water ecosystem. *Heliyon* **9** (2023) e20968. https://doi.org/10.1016/j.heliyon.2023.e20968

Kocour M, Kohlmann K (2011) Growth hormone gene polymorphisms in Tench, *Tinca tinca*. *Aquac* **310**: 298–304. https://doi.org/10.1016/*j.aquaculture*.2010.10.006

Le Gac F, Blaise O, Fostier A, Le Bail PY, Loir M (1993) Growth hormone (GH) and reproduction a review. *Fish Physiol Biochem* **11**: 219–232. https://doi.org/ 10.1007/BF00004569

Li MJ, Liu WS, Luo W, Zhang XQ, Zhu WL, Wang J, Liao LY, Li GH (2017) Polymorphisms and their association with growth traits in the growth hormone gene of yellow catfsh, *Pelteobagrus fulvidraco*. *Aquac* **469**: 117–123. https://doi.org/10.1016/*j.aquaculture*.2016.11.028

Ling L, Liu Y, Zhang X, Aziz T, Shahzad M, Sameeh MY, Wang Y, Cai C, Zhu Y (2023). Effect of Flammulina velutipes polysaccharides on the physicochemical properties of catfish surimi and myofibrillar protein oxidation during frozen storage. *Front Nutr* **10**: 1268580. https://doi.org/10.3389/fnut.2023.1268580

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta DeltaC(T)) method. *Methods* **25**: 402–408. https://doi.org/10.1006/meth.2001.1262

Ma Q, Liu S, Zhuang Z, Lin L, Sun Z, Liu C, Ma H, Su Y, Tang Q (2012) Genomic structure, polymorphism and expression analysis of the growth hormone (GH) gene in female and male half smooth tongue sole (*Cynoglossus semilaevis*). *Gene* **493**: 92–104. https://doi.org/10.1016/j.gene.2011.11.015

Mackay TF (2001) The genetic architecture of quantitative traits. *Annu Rev Genet* **35**: 303–39. https://doi.org/10.1146/*annurev.genet*.35.102401.090633

Marchant TA, Moroz BM (1993) In vitro effects of insulin-like growth factors and related peptides on the uptake of [35S]- sulfate from the goldfish (*Carassius auratus*). *Fish Physiol Biochem* **11**: 393–400. https://doi.org/10.1007/bf00004589

Maures T, Chan SJ, Xu B, Sun H, Ding J, Duan C (2002) Structural, biochemical, and expression analysis of two distinct insulin-like growth factor I receptors and their ligands in zebrafish. *Endocrinol* **143**: 1858–1871. https://doi.org/10.1210/endo.143.5.8768

May D, Alrubaian J, Patel S, Dores RM, Rand-Weaver M (1999) Studies on the GH/SL gene family: cloning of African lungfsh (*Protopterus annectens*) growth hormone and somatolactin and toad (*Bufo marinus*) growth hormone. *Gen Comp Endocrinol* **113**: 121–135. https://doi.org/10.1006/gcen.1998.7185

Milla S, Wang, N, Mandiki, SNM, Kestemont P (2009) Corticosteroids: Friends or foes of teleost fish reproduction? *Comp Biochem Phys* **153**: 242–251. https://doi.org/10.1016/j.cbpa.2009.02.027

Moriyama, S, Dickhoff, WW, Plisetskaya, EM (1995) Isolation and characterization of insulin-like growth factor-I from rainbow trout, *Oncorhynchus mykiss*. *Gen Comp Endocrinol* **99**: 221–229. https://doi.org/10.1006/gcen.1995.1105

Moriyama S, Ayson GF, Kawauchi H (2000) Growth regulation by insulin-like growth factor-I in fish. *Biosci Biotech Bioch* **64**: 1553–1562. https://doi.org/ 10.1271/bbb.64.1553

Mun SH, You JH, Oh HJ, Lee CH, Baek HJ, Lee YD, Kwon JY (2019) Expression patterns of growth-related genes in juvenile red spotted grouper (*Epinephelus akaara*) with different growth performance after size grading. *Dev Reprod* **23**: 35–42. https://doi.org/10.12717/DR.2019.23.1.035

Mullis PE (2005) Genetic control of growth. *Eur J Endocrinol* **152**: 11–31. https://doi.org/10.1530/eje.1.01797

Opazo R, Valladares L, Romero J (2017) Comparison of gene expression patterns of key growth genes between different rate growths in zebrafish (*Danio rerio*) siblings. *Lat Am J Aquat Res* **45**: 766–775. https://doi.org/10.3856/vol45-issue4-fulltext-12

Pal A, Chakravarty AK, Bhattacharya TK, Joshi BK, Sharma A (2004) Detection of polymorphism of growth hormone gene for the analysis of relationship between allele type and growth traits in Karan

fries cattle. *Asian-australas J Anim Sci* **17**: 1334–1337. https://doi.org/10.5713/ajas.2004.1334

Perez-Sanchez, J, Marti-Palanca, H, Le Bail, PY (1994) Seasonal changes in circulating growth hormone (GH), hepatic GH-binding and plasma insulin-like growth factor-I immunoreactivity in a marine fish, gilthead sea bream, *Sparus aurata*. *Fish Physiol Biochem* **13**: 199–208. https://doi.org/10.1007/bf00004358

Purohit GK, Mahanty A, Mohanty BP, Mohanty S (2016) Evaluation of housekeeping genes as references for quantitative real-time PCR analysis of gene expression in the murrel *Channa striata* under high-temperature stress. *Fish Physiol Biochem* **42**: 125–135. https://doi.org/10.1007/s10695-015-0123-0

Quik EH, Van Dam PS, Kenemans JL (2010) Growth hormone and selective attention: A review. *Neurosci Biobehav* Rev **34**: 1137–1143. https://doi.org/10.1016/j.neubiorev.2010.01.001

Rio DC, Ml A, Hannon GJ, Nilsen TW (2010) Purifcation of RNA using TRIzol (TRI reagent). *Cold Spring Harb Protoc* pp 5439. https://doi.org/10.1101/pdb.prot5439

Reinecke M, Bjornsson BT, Dickhoff WW, McCormick SD, Navarro I, Power DM, Gutierrez J (2005) Growth hormone and insulin-like growth factors in fish: where we are and where to go. *Gen Comp Endocrinol* **142**: 20–24. https://doi.org/10.1016/j.ygcen.2005.01.016

Rajesh R, Majumdar KC (2007) A comparative account of the structure of the growth hormone encoding gene and genetic interrelationship in six species of the genus Labeo. *Fish Physiol Biochem* **33**: 311–333. https://doi.org/10.1007/s10695-007-9164-3

Shamblott MJ, Cheng CM, Bolt D, Chen TT (1995) Appearance of insulin-like growth factor mRNA in the liver and pyloric ceca of a teleost in response to exogenous growth hormone. *Proc Natl Acad Sci USA* **92**: 6943–6946. https://doi.org/10.1073/pnas.92.15.6943

Sugita H, Ardiyanti A, Yokota S, Yonekura S, Hirayama T, Shoji N, Yamauchi E, Suzuki K, Katoh K, Roh SG (2014) Effect of single nucleotide polymorphisms in gh gene promoter region on carcass traits and intramuscular fatty acid compositions in Japanese black cattle. *Livest Sci* **165**: 15–21. https://doi.org/10.1016/j.livsci.2014.04.026

Sheridan MA Hagemeister AL (2010) Somatostatin and somatostatin receptors in fish growth. *Gen Comp Endocrinol* **167**: 360–365. https://doi.org/10.1016/j.ygcen.2009.09.002

Tanamati F, Da Silva SCC, Rodriguez MDPR, Schuroff GP, Do Nascimento CS, Del Vesco AP, Gaparino E (2015) GHR and IGF-I Gene expression and production characteristics associated with GH gene polymorphism in Nile Tilapia. *Aquac* **435**: 195–199. https://doi.org/10.1016/j.aquaculture.2014.09.033

Tian C, Yang M, Lv L, Yuan Y, Liang X, Guo W, Song Y, Zhao C (2014) Single nucleotide polymorphisms in growth hormone gene and their association with growth traits in *Siniperca chuatsi*. *Int J Mol Sci* **15**: 7029–7036. https://doi.org/10.3390/ijms15047029

Tymchuk WE, Beckmen B, Devlin RH (2009) Altered expression of growth hormone/insulin-like growth factor – axis hormones in domesticated fish. *Endocrinol* **150**: 1809–1816. https://doi.org/10.1210/en.2008-0797

Vairamani K, Merjaneh L, Casano-Sancho P, Sanli ME, David A, Metherell LA, Savage MO, Sanchez del Pozo J, Backeljauw PF, Rosenfeld RG Aisenberg J (2017) Novel dominant-negative GH receptor mutations expands the spectrum of GHI and IGF-I deficiency. *J Endocr Soc* **1**: 345–358. https://doi.org/10.1210/js.2016-1119

Wasim MP (2007) Issues, growth and instability of inland fish production in Sindh (Pakistan): Spatial – Temporal Analysis. *Pakistan Economic and Social Review* 203–230

Wu Y, Pan AL, Pi JS, Pu YJ, Du JP, Liang, ZH, Shen J (2012) One novel SNP of growth hormone gene and its associations with growth and carcass traits in ducks. *Mol Bio Rep* **39**: 8027–8033. https://doi.org/10.1007/s11033-012-1649-1

Xu SH, Bao WB, Huang J, Cheng JH, Shu JT, Chen GH (2007) Polymorphic analysis of intron 2 and 3 of growth hormone gene in duck. *Hereditas* **29**: 438–442. https://doi.org/10.1360/yc-007-0438

Yi TL, Fang L, Liang XF, Sun LF, Li J, Luo XN, Guo WJ, Dou, YQ, Sun, J (2015) Characterization of microsatellite markers and their correlations with growth traits in mandarin fish (*Siniperca Chuatsi*). *Genet Mol Res* **14**: 8926–8934. https://doi.org/10.4238/2015.August.7.1

Zhang D, Shao Y, Jiang S, Li J, Xu X (2009) *Nibea Coibor* Growth hormone gene: its phylogenetic significance, microsatellite variation and expression analysis. *Gen Comp Endocrinol* **163**: 233–241. https://doi.org/10.1016/j.ygcen.2009.03.030

Zhong H, Zhou Y, Liu S, Tao S, Long Y, Liu Z, Zhang C, Duan W, Hu J, Song C, Liu Y (2012) Elevated expressions of GH/IGF axis genes in triploid crucian carp. *Gen Comp Endocrinol* **178**: 291–300. https://doi.org/10.1007/s11033-010-0522-3