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EFFECT OF ARSENIC ON THE BIOLOGICAL TRAITS OF THE MAJOR CARP, ROHU (*Labeo rohita*)

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Abstract
Arsenic is a highly toxic metalloid that can have detrimental effects on farmed aquatic species, negatively impacting growth, metabolism, immunity and overall wellbeing of fish. The Indian major carp, Rohu (*Labeo rohita*) is a major freshwater aquaculture species that faces various production related issues associated with water quality parameters. The current study was designed to elucidate the effects of three different doses of arsenic (As) (T_1 = 1 µg/L, T_2 = 2 µg/L, and T_3 = 3 µg/L) on the physiological (growth and O_2 consumption), biochemical (blood cell counts), and genetic (expression levels of three selected genes) responses of Rohu. This study revealed significant (P<0.05) biologicl abnormalities and deformities in arsenic-exposed Rohu carp. The growth rate of fish decreased with increasing arsenic concentrations. O_2 consumption rates of fish were increased (1.6 – 2 fold) with increasing experimental arsenic concentrations but blood cell counts were in a declining trend. Expression levels of the three selected genes showed arsenic dose specific differential changes; higher expression at control condition (1.5 – 2.1 fold) while lower expression at the treatment conditions. Results of this study clearly point out that different doses of arsenic impose stress at different orders of magnitude on the experimental Rohu individuals. The findings of the present study suggest that arsenic pollution significantly impacts the physiological (growth, development, metabolism and survivability), biochemical (hematological parameters) and molecular mechanisms of this economically important fish (*L. rohita*). Therefore, it is an imperative to maintain the optimum water quality (pollutant free) in the farming environments.

Key words: Major carp, arsenic, climate change

Introduction

Environmental pollution, particularly water pollution, has emerged as a significant global concern. Water pollution not only has a negative impact on growth, survival, and reproduction of aquatic animals, but it also has a negative impact on human life due to bioaccumulation and subsequent bio-magnification (Sabbir et al., 2010; Dai et al., 2014; Rahi et al., 2020). For fishes, the early developmental stages are extremely vulnerable to any type of stressors including water pollution (Mridul et al., 2024). Due to various industrial activities (agriculture, different types of industrialization, domestic sewerage and navigation), different types of pollutants (e.g. heavy metals) are accumulating in the water bodies at an alarming rate (Muhammad et al., 2023). Developmental and physiological processes of fish, such as- organ development, breeding and spawning, are severely affected by heavy metal contamination (such as- arsenic, cadmium, lead, mercury, etc.), which lowers the quantity and quality of their offspring (Jezierska et al., 2009; Zeehad et al., 2024). *Labeo rohita*, commonly known as the Rohu, is a freshwater fish species native to the Indian subcontinent. Approximately 8% (345,000 metric tons) of Bangladesh's total fish production comes from this species, with a market value of about 69,000 million taka (DoF, 2020). Due to its faster growth rate (reaching market size within 6 months post stocking under suitable conditions), delicious taste, and good domestication ability, ability to accept formulated feed, disease resistance, and higher market price, Rohu has proven efficiency as a suitable species for aquaculture (Rahi & Shah, 2012; Mridul et al., 2024). Although Bangladesh's aquaculture production is steadily improving, the natural abundance of various fish species, including Rohu, has decreased significantly (Akram et al., 2023). Natural production shrinkage can pose a serious threat to the sustainable aquaculture development of Bangladesh by drastically reducing population size and lower genetic variation (risk of inbreeding). Various industrial activities (agriculture, different types of industrialization, domestic sewerage and navigation) and different types of pollutants (e.g. heavy metals) are the main reasons for the reduced abundance of different fish species in the natural freshwater bodies of Bangladesh (Nath et al., 2008; Islam et al., 2011; Rahi & Shah, 2012; Rahi et al., 2022). Arsenic is one of the most poisonous heavy metals in the aquatic environment, significantly contaminating different water bodies (Ratnaike, 2003; Oremland & Stolz, 2003). It is a metalloid toxicant that is commonly found in rivers, canals, ponds, groundwater, lakes, and seawater as a result of the uncontrolled influx of industrial waste and pesticides into the aquatic environment (Kumari et al., 2016). Arsenic has been identified by the World Health Organization (WHO) as one of the most hazardous substances to human health (Babich & Van Beneden, 2019). Many countries, including Chile and Bangladesh, have documented arsenic levels of up to 800 and 2500 ppm as the maximum limit of tolerance (Naujokas et al., 2013). Rohu commonly lives in native aquatic habitats. Therefore, this fish regularly experience exposure to arsenic by a number of anthropogenic activities, including its release through industrial activities (Vutukuru et al., 2007; Shah et al., 2011; Sabbir et al., 2017). High levels of arsenic exposure to human being are directly linked to a wide range of illnesses, including liver disorders, cardiovascular disease, skin and lung cancer (Kundu et al., 2011; Gong & O'Bryant, 2012). The effect of this toxicant can bring about biochemical and physiological changes in fish, causing them to grow and develop at a slower rate (Beyers et al., 1999; Han et al., 2019; Rahi et al., 2021). Tissue histo-pathological research is a useful method for assessing the effects of environmental pollutants on the individual important organs of fish under laboratory conditions (Bose *et al*., 2013; Javed & Usmani, 2017). The extent of pathological alterations in the various fish organs, however, is determined by the quantity of the dosage and length of exposure to contaminants (Rahi, 2017; Afroz et al., 2021). Histopathological investigations can also assist in establishing the links between various biological processes and pollutant exposure (Selvi & Ilavazhahan, 2012).

Investigating the impact of arsenic on growth, survival, and oxygen (O_2) consumption, blood cell counts and changes in relative expression of selected candidate genes represent some biological markers to examine the intensity of stress (Aziz et al., 2017; Moshtaghi et al., 2018; Rahi et al., 2023) and also the overcome those stress tolerance abilities of Rohu. Therefore, the effect of arsenic can bring biochemical and physiological changes in rohu but these aspects have not been tested with adequate coverage. Considering the lack of information about the effect of arsenic toxicity on commercially important and popular fish species, Rohu in Bangladesh, the present experiment has been designed to explore the effect of arsenic on the selected biological traits of Rohu (*Labeo rohita*).

Materials and Methods

Sample Collection and Maintenance

Fingerlings of Rohu (≈0.4 g of mean body weight) were collected from a commercial hatchery for this study (nursery rearing ponds of the 'Fish Seed Multiplication Farm', Gollamari, Khulna). Fishes were maintained under four different doses of arsenic (As) including a control (no arsenic). Different experimental conditions were maintained as: 0 µg/L (as Control), 1 µg/L (T_1), 2 µg/L (T_2) and 3 µg/L (T_3). In total, 12 experimental glass tanks (25 L) were maintained for this experiment including three replicated tanks for each experimental condition. In each experimental tank, 25 fish individuals were maintained with continuous aeration (75 fish per treatment). At first, a stock solution was prepared by mixing arsenic tri-chloride ($AsCl_3$) with water. Appropriate amount of the solution was added in each tank to achieve the target As doses. Fishes were maintained under the As doses for 30 days.

Growth and Survival

Mean body weight of experimental Rohu was measured at every 10 days interval. For growth evaluation, 30 individuals were randomly sampled from each treatment (10 from each replicate tank). Survival rates were estimated by deducting the number of individuals from the beginning to the end of this experiment. Further growth related parameters were measured according to the following equations (Zeynali et al., 2020; Rahman et al., 2022):

$$DWG\ (\%) = \{(BW_f - BW_i)/ (BW_i \times t)\} \times 100$$

$$SGR\ (\%) = \{(\ln BW_f - \ln BW_i)/t\} \times 100$$

$$FI\ (g\ fish^{-1}days^{-1}) = (\text{dry diet given - dry remaining diet recovered}) / \text{number of fish}$$

Here, DWG = daily weight gain, BW_f = final body weight, BW_i = initial body weight, t = total experimental time (30 days), SGR = specific growth rate, FCR = feed conversion ratio and FI = Feed Intake.

Rates of O_2 Consumption

Experimental Rohu were sampled at 8 different time intervals to measure the As treatment specific changes in O_2 consumption rates. The sampling times for measuring O_2 consumption rates were at Day 1 (immediately after achieving the target As doses), Day 2, Day 3, Day 4, Day 5, Day 10, Day 20 and Day 30. Three replicated fish (one from each tank) were utilized for estimating O_2 consumption rates. O_2 consumption rates were measured in a 250 mL flow respirometric chamber (Q-Box Aqua Respiratory System, Qubit, Canada) according to the methods outlined in Rosas et al. (2001), Rahi et al. (2022) using the following equation:

$$O_2 \text{ consumption} = [O_2 \text{ en} - O_2 \text{ ex}] \text{ x FR}$$

Here, water flow rate (FR) = 1.5 Lh^{-1} in the respirometric chamber, O_2 en = amount of O_2 at the entry of respirometric chamber and O_2 ex = amount of O_2 during exit.
After measuring the O_2 consumption, experimental fishes were quickly weighed, in triplicates for each treatment, to estimate the O_2 consumption rates (mg O_2 $hr^{-1}g^{-1}$).

Blood Cell Counts
Three replicate fish were collected from each experimental condition for counting the number of blood cells. From each fish, 50 µL of blood was collected using heparinized micro-injection and was immediately transferred in eppendorf tubes containing an equal volume (50 µL) of 20 mM EDTA. Following this step, 100 µL of 10% neutral buffered formalin was added in each of the blood samples and maintained at ambient temperature for 30 minutes to fix the samples. Samples were then serially diluted for 2, 4, 8, 16, and 32 times using ice-cold phosphate buffered saline (PBS, 20 mM, pH 7.2) (Witeska *et al.*, 2022; Akram et al., 2023). Finally, the total number of normal blood cells was counted using a hemocytometer (Boeco, Hamburg, Germany) and checked under a microscope (SOLARIS-TLED, Rome, Italy) at 100x magnification.

Gene Expression Study
Due to the small size of experimental fish, whole individuals of Rohu were required to investigate the expression pattern of different genes. As mentioned earlier, Sampling was performed at 8 different times, where Day 1 denotes sampling immediately after exposure to various arsenic levels. At each sampling time, three replicate individuals were collected from each arsenic treatment. Total RNA was extracted from the experimental fish samples using the TRIzol/chloroform extraction method, followed by RNA purification using a commercial RNA extraction kit (Qiagen, Germany) according to the manufacturer's protocol. Total RNA integrity (quality and quantity) for each sample was evaluated using 2% agarose gelelectrophoresis and a NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA). The high quality RNA samples were preserved at -80ºC for subsequent use. Total RNA samples (using 1 µg RNA for each sample) were used for complementary DNA (cDNA) synthesis by using the SensiFAST cDNA synthesis kit (Bioline, UK) according to the manufacturer's protocol and preserved at -20ºC for further use.

Three different genes with distinct functional roles were used for the RT-qPCR-based gene expression study, including insulin like growth factor (IGF-I which is involved with growth regulation, Glycerol-3-phosphate (G-3-P) and Ghrelin (these two genes are involved with metabolic activities). Elongation factor 1 alpha (EF 1α) was chosen as the reference gene, as its suitability has been demonstrated numerous times in other fish studies (Sahu et al., 2015; Islam et al., 2020; Zeynali et al., 2020).

The candidate genes were chosen due to their functional roles elucidated in previous research (Sahu et al., 2015; Islam et al., 2020). Sequences of the candidate genes were obtained from an earlier study (Sahu et al., 2015) to design gene-specific primers (Table 1) using the Primer3 software (Untergasser et al., 2012). RT-qPCR reactions were performed in 20 µL mixtures including 10 µL of 2x SensiFAST SYBR No-ROX Mix (Bioline, UK), 3 µL of ultra-pure water, 5 µL of template cDNA, and 1 µL each of forward and reverse primer. Three technical replicates of each sample were used in the RT-qPCR procedures, which were carried out on a real-time PCR system (Bio-Rad, California, USA). At the end of the reaction, a standard melt-curve analysis was performed to confirm the amplification of a single qPCR product (Moshtaghi et al., 2017; Aziz et al., 2018; Rahi et al., 2019). Finally, data obtained from the RT-qPCR were

analyzed (relative expression level of each gene) using the ΔΔCt method (Pfaffi, 2001; Kokou et al., 2019) according to the following equation:

$$\text{Relative Gene Expression (R)} = 2^{-[\Delta Ct\ \text{Target Gene} - \Delta Ct\ \text{Reference Gene}]}$$

Table 1: Specific details of the primers for the selected candidate genes (Sahu *et al.*, 2015; Shahjahan *et al.*, 2021) used in this study (F = Forward, R = Reverse).

Candidate Gene	Primer Sequence (5′ – 3′)	Product Length (bp)	Annealing Temp.
Insulin like growth factor I (IGF-I)	**F:** CCCGGGGTCAAAATGCAGCT	120	57ºC
	R: GGGGTAACTCAGGCCACGGA		
Glycerol-3-phosphate (G-3-P)	**F:**CGTCCTGTTCACTGCACCCAG	112	60ºC
	R:ATGCCACAGCAGACGTCGCT		
Ghrelin	**F:** TGCAGGTCTCTGTGGTGGTG	130	61ºC
	R:ACAGCTGGATGCTGGGCAGT		
Elongation factor 1 alpha (EF 1α)	**F:** TTCGAGCAGGAGATGGGCACTG	114	60ºC
	R: GCATCCTGTCAGCAATGCCA		

Statistical Analysis

Different types of data were checked for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests, respectively using the software package SPSS (Version 23). Results were also evaluated for one- and two- way analysis of variance (ANOVA) using 5% level of significance ($P < 0.05$). Treatments and sampling times were considered as independent variables for two-way ANOVA while comparisons were made only between treatments for one-way ANOVA. The dependent variables were different physiological (growth and O_2 consumption), biochemical (number of blood cells) and genetic (gene expression) parameters. Comparisons between the means of different parameters were evaluated using the Tukey HSD test. Results were also presented in the tables and graphs as mean ± standard error (SE).

Results

Growth and Survival Performance

Different doses of arsenic significantly altered growth ($F(4, 120) = 126.3$, $P = 0.00$) and survival performance ($P<0.05$) of experimental Rohu individuals (Table 2 and Figure 1). Initially, no significant difference was observed among control and the treatment groups. Significantly higher growth and survival performance ($P<0.05$) were observed at the control condition compared to the treatment groups throughout the experiment. The overall analysis has revealed a significant effect of arsenic on various growth related parameters (DWG, SGR and FI) and survival rate of the experimental fishes (Table 2). Up to the 10^{th} day, there was no significant difference between the treatments (T_1 and T_2), while T_3 showed significant difference with the other treatments and also control (Figure 1). T_1 showed significantly higher growth over the T_2 and T_3 from the 10^{th} day to the end of the study.

Table 2: Effects of Arsenic (As) doses on growth parameters (mean ± SE) of Rohu (*Labeo rohita*). Different superscript letters (a, b, c, d) in the same row indicate significant differences (P< 0.05) between experimental conditions.

Growth Parameters	Control	T_1	T_2	T_3
Initial Weight (BW_i) (g)	0.4^a ±0.08	0.4^a± 0.07	0.4^a± 0.09	0.4^a± 0.08
Final Weight (BW_f) (g)	2.04^a± 0.6	1.76^b± 0.4	1.67^b± 0.3	1.29^d± 0.5
Daily Weight Gain (DWG) (%)	13.66^a± 1.6	11.33^b± 1.3	10.58^c± 1.4	7.42^d± 1.5
Specific Growth Rate (SGR) (%)	5.43^a± 0.6	4.94^b± 0.7	4.76^b± 0.5	3.90^d± 0.4
Feed Intake (FI) (g g^{-1} day^{-1})	0.151^a	0.156^b	0.158^b	0.161^b
Survival Rate (%)	100^a	91^b	83^c	71^d

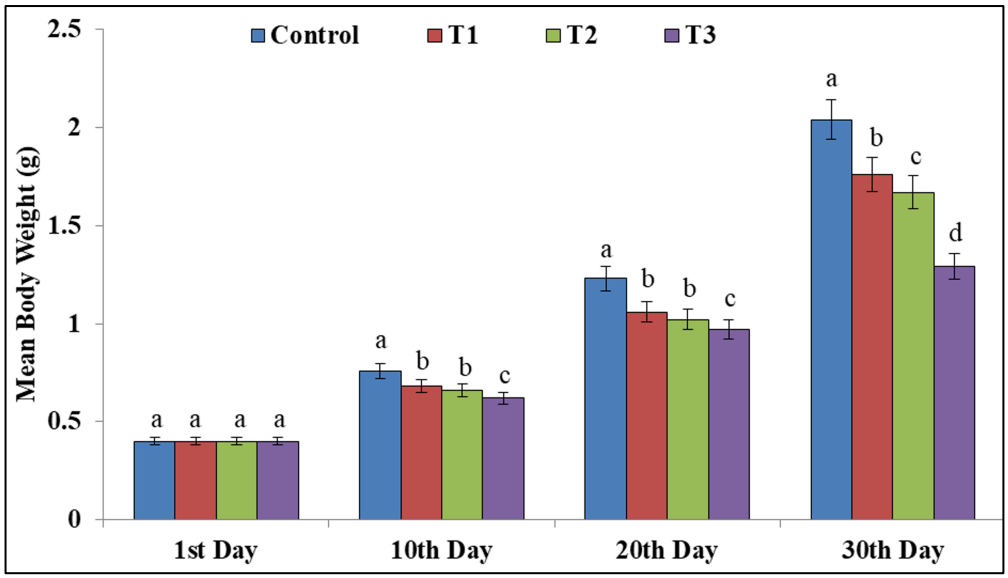

Figure 1: Mean body weight (g) (±S.E.) of the experimental Rohu (Labeo rohita) at every 10 days interval. Different letters above the bar indicate significant difference at 5% level of significance.

Rates of O_2 Consumption

This study was revealed a significant effect (F (8, 96) = 98.5, P = 0.00) of different arsenic doses on O_2 consumption rates of the experimental Rohu. Throughout the entire experiment, the O_2 consumption rate remained constant in control group while it was found to vary significantly for the treatment groups (P<0.05) across the sampling time. At the beginning of this experiment, no significant differences were found among treatments (Figure 4).

From Day 2 to the end, the treatment groups showed significantly higher O_2 consumption rates than the control. O_2 consumption rate in treatment groups was in an increasing trend up to Day 3, followed by a declining phase up to 4th day and then it remained stable for the remaining time. Highest O_2 consumption rate was found in T_3.

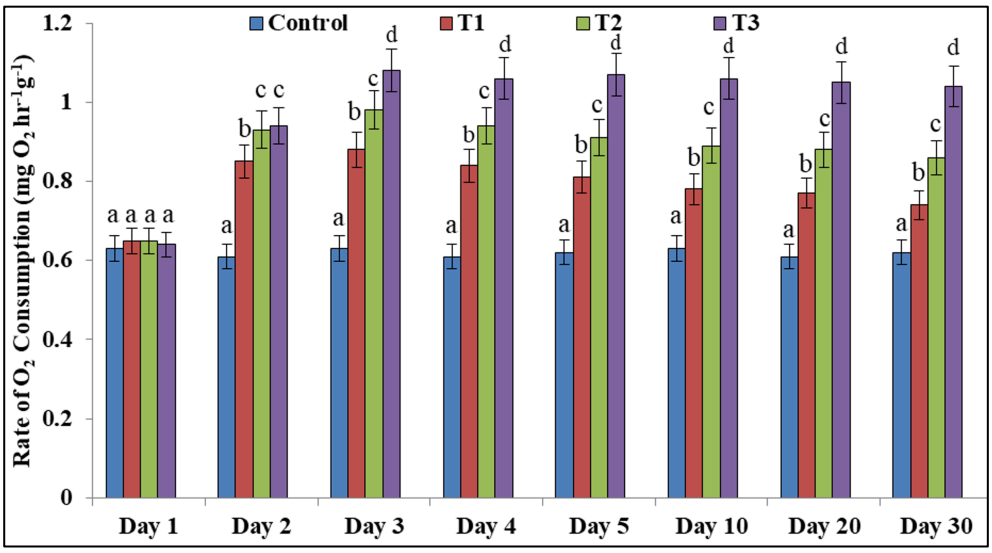

Figure 2: Arsenic induced changes (Mean ± S.E.) in the rate of O_2 consumption in experimental Rohu (*Labeo rohita*) individuals across the sampling times. Different letters above the bar indicate significant difference at 5% level of significance.

Blood Cell Counts

The study was revealed a significant effect (F (8, 96) = 101.8, P = 0.001) of different arsenic doses on the number of blood cells of the experimental Rohu (Figure 3). From the 2nd day until the end of the experiment, fish in the control group had significantly higher numbers of blood cell counts (P<0.05) compared to the arsenic treatments. From beginning to the end, no significant differences for blood cell counts were found among the treatment groups. Up to the 5th day, the number of blood cells was in a declining trend, but after that point, there was a stable trend up to the end (Figure 3).

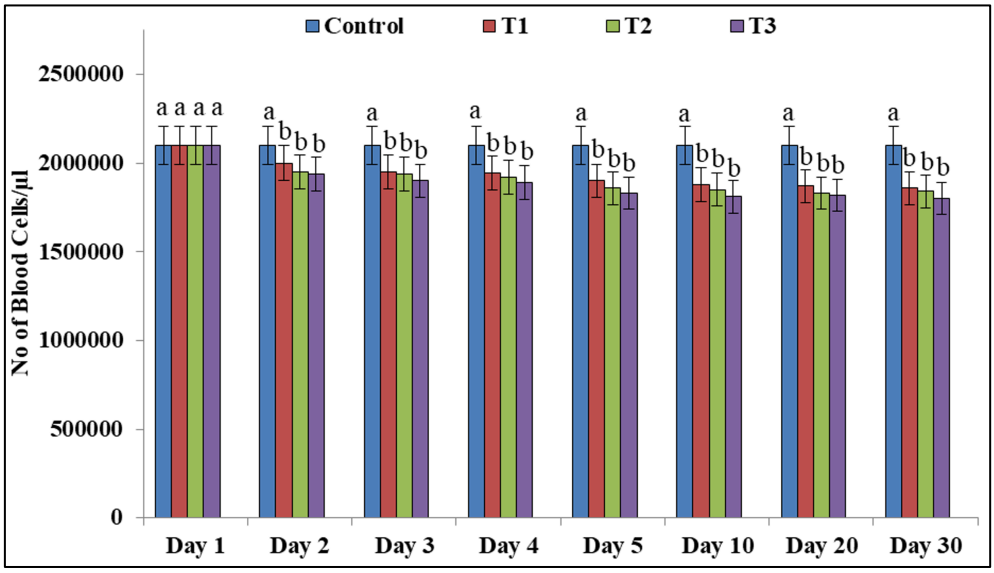

Figure 3: Arsenic induced changes in the number of blood cell counts for experimental Rohu (*Labeo rohita*) across the sampling times. Different letters above the bar indicate significant difference at 5% level of significance.

Relative Expression Levels of Target Candidate Genes

The study was revealed a significant effect (P<0.05) of different arsenic doses on the relative expression levels of growth regulatory genes (Insulin like growth factor I (IGF-I)) of the Rohu

fingerlings (Figures 4). The highest levels of IGF-I expression pattern were observed for the control group compared to the treatment groups. No significant differences were observed between the control and arsenic treatments for the IGF-I gene initially (1st day), but after that point, the control group revealed significantly higher expression levels for the entire experimental period. After the 1st day, T_1 and T_2 showed significantly higher IGF-I expression levels over T_3 for the remaining time frame (Figure 4), while no significant differences were found between T_1 and T_2 for the IGF-I expression throughout the experiment.

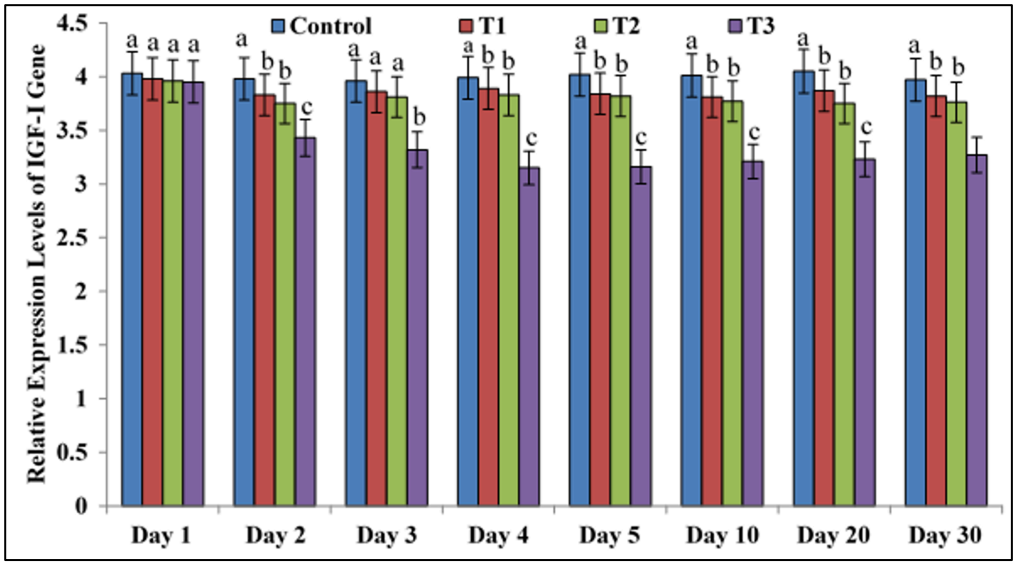

Figure 4: Changes in relative expression levels of insulin like growth factor I (IGF-I) gene for experimental Rohu (*Labeo rohita*) across the sampling times. Different letters above the bar indicate significant difference at 5% level of significance.

The study revealed a significant effect ($P<0.05$) of different arsenic doses on the relative expression levels of metabolic gene (Glycerol-3-phosphate (G-3-P)) of the Rohu fingerlings (Figures 5). At the beginning of this experiment, no significant differences were observed between the control and arsenic treatments for the G-3-P gene initially (1st day), but after that point, the control group revealed significantly higher expression levels for the entire experimental period. Up to the 2nd day, there were no significant differences between the treatments (T_1 to T_3), but after that point, there were significant differences ($P<0.05$) between the treatment groups (Figure 5).

The expression levels of the neuropeptide ghrelin revealed significant differences ($P<0.05$) with arsenic treatments (Figure 6).

At the start of this experiment, no significant differences were found between the control and arsenic treatments for the ghrelin initially (1st day), but after that point, the control group revealed significantly higher expression levels than the other three treatment groups for the entire experimental period. After the 1st day, T_1 and T_2 showed significantly higher ghrelin expression levels over T_3 for the remaining time frame (Figure 6), while no significant differences were found between T_1 and T_2 for the ghrelin expression throughout the experiment.

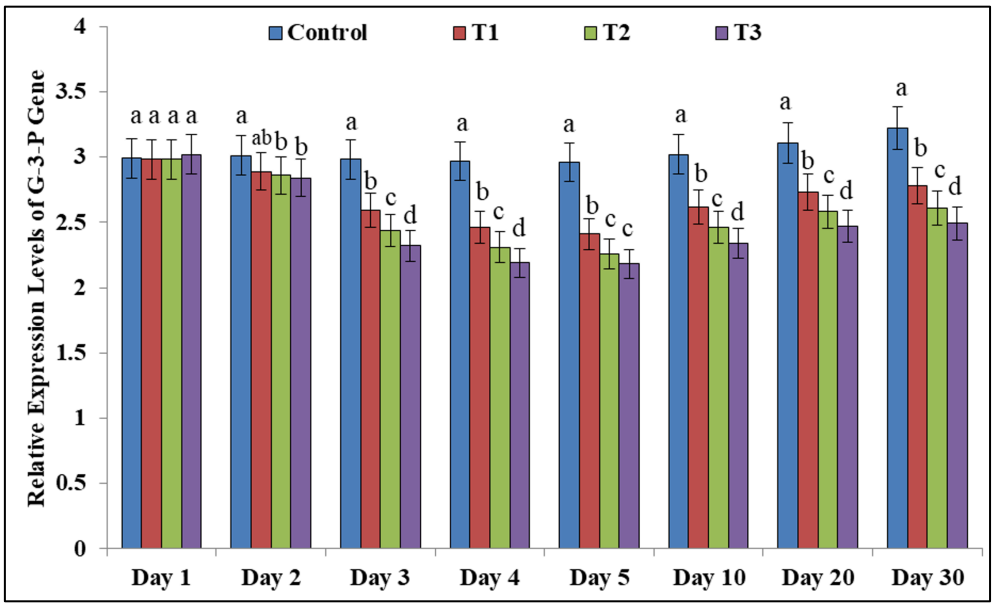

Figure 5: Changes in relative expression levels of Glycerol-3-phosphate (G-3-P) gene for experimental Rohu (*Labeo rohita*) across the sampling times. Different letters above the bar indicate significant difference at 5% level of significance.

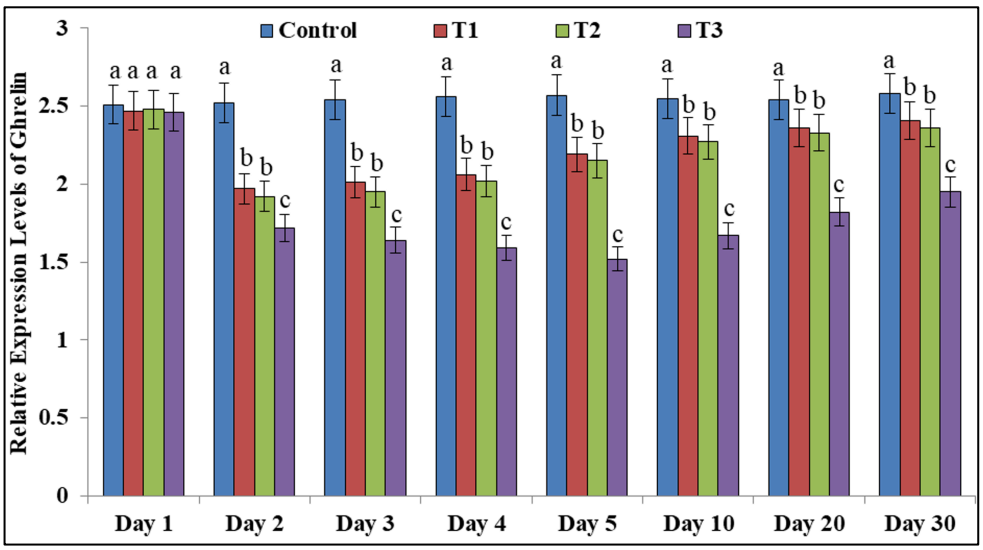

Figure 6: Changes in relative expression levels of ghrelin for experimental Rohu (*Labeo rohita*) across the sampling times. Different letters above the bar indicate significant difference at 5% level of significance.

Discussion

Growth and Survival Performance

Results from this study have shown that the different doses of arsenic lead to a significant effect on experimental Rohu fish growth parameters and survival performance. Significant differences in growth and survival performance between the experimental groups (Table 2 and Figure 1) clearly indicate the adverse effect of arsenic on the growth performance of Rohu (*Labeo rohita*). The study has revealed significant differences in growth and survival performance among the treatments (T_1 – T_3) which indicate different doses of arsenic affects different levels of stress on Rohu and thereby, provided differential growth and survival. Mean body weight of

experimental Rohu was reduced with the increasing exposure time and concentration of the arsenic. A similar result was found by some other researchers when Rohu fish were treated with different pollutants (Hayat et al., 2007; Banerjee et al., 2015). When fish were exposed to a sub-lethal quantity of manganese for 30 days, their growth was significantly reduced in *Labeo rohita*, *Catla catla*, and *Cirrhina mrigala* (Ali et al., 2008; Banerjee et al., 2015). In general, the relationship between fish weight and heavy metal concentration is opposite, whereas mortality shows a positive relationship with heavy metal concentration (Hussain et al., 2010; Islam et al., 2014). The condition factor is another index that determines the general wellbeing of fish. A higher value of the condition factor shows a better environmental situation, whereas a lower value indicates a poor environmental condition (Javed & Usmani, 2017). Nevertheless, very few researchers have utilized the condition factor as a biomarker of environmental pollution. A previous work also revealed that the condition factor is not only affected by pollutants but also some other factors are also responsible, such as food availability, temperature, etc. (Javed and Usmani, 2017; Rahi et al., 2017). Therefore, arsenic has detrimental effects on fish development, growth and mortality (Ahmed et al., 2013; Foley et al., 2016). Similarly, in this experiment, Rohu fish mortality was significantly affected by arsenic exposure because mortality was increased with the increasing arsenic concentration and we found the highest mortality in T_3, followed by T_2.

Changes in the Rate of O_2 Consumption

Changes in the rate of O_2 consumption show the magnitude or strength of available stress (Fromm & Gillette, 1968). Higher rate of O_2 consumption potentially point out that organisms are under increased intensity of stress (Foss et al., 2003). In this experiment, O_2 consumption rates for experimental Rohu were observed to be in an increasing trend with increasing experimental arsenic concentration (Figure 2). From beginning to the end of this experiment, stable O_2 consumption rates and significantly higher growth of control group over the treatments indicate no imposed stress. Higher rates of O_2 consumption from T_1 to T_3 compared to the control group point out higher levels of imposed stress on treatment groups of Rohu. Fish take higher rates of O_2 under stressful condition to meet the growing demand of energy for counterbalancing the adverse effects of stressors (Islam et al., 2015; Rajendiran et al., 2016; Rahi et al., 2020). The highest rates of O_2 consumption at T_3 show the maximum level of stress was imposed at this treatment while moderate level was imposed at T_2 and minimum level was for T_1.

Blood Cell Counts

Relatively higher and stable number of blood cells shows no imposed stress for the control groups. Number of blood cells indicated a generalized decreasing trend with increasing arsenic levels (Figure 3). Arsenic directly damages the cells of fish gill lamellae and blood cells that restrict O_2 transportation (Randall, 1990). Therefore, O_2 requirements increase when fish are exposed to arsenic (Li *et al*., 2014). In this regard, fishes tend to reduce the number of blood cells because of cell lysis for counterbalancing the adverse effects of arsenic (Wedemeyer, 1996). Blood cells break down and number decreases up to a particular time and then remains stable due to arsenic challenge (Cheng et al., 2015; Rahi et al., 2021). This likely describes the reason for the reduction of blood cells for the arsenic challenged fish samples.

Relative Expression Levels of Target Candidate Genes

Insulin like growth factor I (IGF-I) is known to have important functional roles in growth regulation in different fish species while Glycerol-3-phosphate (G-3-P) is related with metabolic activities and the ghrelin (neuropeptide) triggers growth related processes by stimulating appetite (Li et al., 2016; Aziz et al., 2018; Triantaphyllopoulos et al., 2020;

Loughland & Seebacher, 2020). Therefore, any environmental stressors can decrease the expression of growth genes that will ultimately cause slower growth performance (Sinha et al., 2012; Aziz et al., 2017; Casu et al., 2017; Moshtaghi et al., 2017; Zarantoniello et al., 2021). Previous studies have shown that arsenic has detrimental effects on fish development, growth and genetic expression (Hayat et al., 2007; Ahmed et al., 2013; Banerjee et al., 2015; Foley et al., 2016; Han et al., 2019). Fish exposed to high levels of arsenic experience changes in body physiology, including effects on growth, ion exchange, immune system and gene regulation (Pedlar et al., 2002; Datta et al., 2009; Rahi et al., 2018; Rogl et al., 2018). In this experiment, the highest levels of IGF-I expression pattern were observed for the control group compared to the treatment groups, whereas experimental arsenic levels significantly decreased the expression levels of IGF-I gene (Figure 4) indicating adverse effects of arsenic on this growth regulatory gene. Similarly, expression of ghrelin (the neuropeptide that governs food intake or appetite in fishes) was also reduced in arsenic treatment groups (Figure 6), indicating the adverse effect of experimental arsenic doses on the expression levels of this essential neuropeptide. Therefore, significantly higher growth was observed at the control condition compared to the treatment groups throughout the experiment.

Conclusion

In the current study, Rohu (*Labeo rohita*) fingerlings were exposed to three different doses of arsenic that were examined for physiological (growth and O_2 consumption), biochemical (blood cell counts), and genetic (expression levels of three target genes) responses. The study showed that experimental arsenic treatments significantly altered growth, survival, O_2 consumption, blood cell counts and gene expression pattern. Growth and survival were reduced, as well as blood cell count was also affected by arsenic. Arsenic and O_2 consumption have a strong relationship, pointing out that Rohu individuals consume more O_2 to fulfill their increasing energy demands as a result of arsenic stress. The study also concludes the detrimental effects of arsenic on genetic expression. The findings of this study clearly show that arsenic has an adverse role on the overall biological traits of Rohu. Even lower doses of arsenic can put on adequate stress and make the fish vulnerable to different disease causing agents. Therefore, farming environments must be maintained with optimum water quality for improving aquaculture productivity.

Ethical approval

The animal study protocol was approved by the "Animal Ethics Committee" of KHULNA UIVERSITY (KUAEC-2021/09/21 and date of approval: 21/09/2021).

Informed consent

Not available

Data availability statement

The authors declare that data can be provided by corresponding author upon reasonable request.

Conflicts of interest

There is no conflict of interests for publishing this study.

Funding organizations

No funding available for this study.

Contribution of authors

Maliha Zaman Lema : Conceptualization, data curation, formal analysis, methodology, software, validation and writing original draft

Md. Fahad Al Zobayer : Data curation, formal analysis, software, visualization and writing original draft

Wasim Akram : Conceptualization, data curation, formal analysis, methodology, reviewing and editing

Fatema Tuz Zahura Anti : Data curation, formal analysis, validation and visualization

Md. Lifat Rahi : Conceptualization, funding, investigation, methodology, project administration, resources, supervision, review and editing

All authors have read and agreed to the published version of the manuscript.

References

Afroz, K. B., Shah, M. S., Salin, K. R. & Rahi, M. L. (2021). Growth and survival of diploid and triploid bata, *Labeo bata* (Hamilton, 1822). *Aquaculture Fish and Fisheries, 1*(1), 42-50.

Ahmed, K., Mamun, H. A., Parvin, E., Akter, M. S., & Khan, M. S. (2013). Arsenic induced toxicity and histopathological changes in gill and liver tissue of freshwater fish, tilapia (*Oreochromis mossambicus*). *Experimental and Toxicologic Pathology, 65*, 903–909.

Akram, W., Tabassum, M., Rahi, M.L. 2023. Cellular, Physiological, and Biochemical Basis of Adaptive Response to Variable Osmotic Environments by the River Shad, *Tenualosa ilisha*. *Journal of Applied Ichthyology, 4910938* (1-12).

Ali, M. Y., Sabbir, W., Rahi, M. L., Chowdhury, M. M. R. & Faruque, M. O. (2008). Quality changes in shrimp (*Penaeus monodon*) stored at ambient temperature in plastic and bamboo basket. *International Journal of Animal and Fisheries Science, 1*(1), 7-13.

Aziz, D., Nguyen, T. V., Rahi, M. L., Hurwood, D. A., & Mather, P. B. (2017). Identification of genes that potentially affect social dominance hierarchy in adult male giant freshwater prawns (*Macrobrachium rosenbergii*). *Aquaculture, 476*, 168-183.

Aziz, D., Rahi, M. L., Hurwood, D. A., & Mather, P. B. (2018). Analysis of candidate gene expression patterns of adult male *Macrobrachium rosenbergii* morphotypes in response to a social dominance hierarchy. *Hydrobiologia, 825*(1), 121-136.

Babich, R., & Van Beneden, R. J. (2019). Effect of arsenic exposure on early eye development in zebrafish (*Danio rerio*). *Journal of Applied Toxicology, 39*(6), 824-831.

Banerjee, S., Mitra, T., Purohit, G. K., Mohanty, S., & Mohanty, B. P. (2015). Immunomodulatory effect of arsenic on cytokine and HSP gene expression in *Labeo rohita* fingerlings. *Fish & Shellfish Immunology, 44*, 43-49.

Beyers, D. W., Rice, J. A., Clements, W. H., & Henry, C. J. (1999). Estimating physiological cost of chemical exposure: Integrating energetics and stress to quantify toxic effects in fish. *Canadian Journal of Fisheries and Aquatic Sciences, 56*, 814-822.

Bose, M. J., Ilavazhahan, M., Tamilselvi, R., & Viswanathan, M. (2013). Effect of heavy metals on the histopathology of gills and brain of fresh water fish *Catla catla*. *Biomedical and Pharmacology Journal, 6*, 99-105.

Casu, F., Watson, A. M., Yost, J., Leffler, J. W., Gaylord, T. G., Barrows, F. T., & Bearden, D. W. (2017). Metabolomics analysis of effects of commercial soy-based protein products in red drum (*Sciaenops ocellatus*). *Journal of Proteome Research, 16*(7), 2481-2494.

Cheng, C. H., Yan, F. F., Ling, R. Z., Liao, S. A., Miao, Y. T., Ye, C. X., & Wang, A. L. (2015). Effects of ammonia exposure on apoptosis, oxidative stress and immune response in puffer fish (*Takifugu obscurus*). *Aquatic Toxicology, 164*, 61-71.

Dai, Y. J., Jia, Y. F., Chen, N., Bian, W. P., Li, Q. K., Ma, Y. B., Chen, Y. L., & Pei, D. S. (2014). Zebra fish as a model system to study toxicology. *Environmental Toxicology and Chemistry, 33*, 11-17.

Datta, S., Ghosh, D., Saha, D. R., Bhattacharaya, S., & Mazumder, S. (2009). Chronic exposure to low concentration of arsenic is immunotoxic to fish: Role of head kidney macrophages as biomarkers of arsenic toxicity to *Clarias batrachus*. *Aquatic Toxicology, 92*, 86-94.

DoF (Department of Fisheries). (2020). *National Fish Week, Compendium Book* (In Bengali). Ministry of Fisheries and Livestock, Department of Fisheries (pp. 86-97). Dhaka, Bangladesh.

Foley, C. J., Bradley, D. L., & Höök, T. O. (2016). A review and assessment of the potential use of RNA:DNA ratios to assess the condition of entrained fish larvae. *Ecological Indicators, 60*, 346–357.

Foss, A., Vollen, T., & Øiestad, V. (2003). Growth and oxygen consumption in normal and O_2 supersaturated water, and interactive effects of O_2 saturation and ammonia on growth in spotted wolffish (*Anarhichas minor*, Olafsen). *Aquaculture, 224*(1-4), 105-116.

Fromm, P. O., & Gillette, J. R. (1968). Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (*Salmo gairdneri*). *Comparative Biochemistry and Physiology, 26*(3), 887-896.

Gong, G., & O'Bryant, S. E. (2012). Low-level arsenic exposure, AS3MT gene polymorphism and cardiovascular diseases in rural Texas counties. *Environmental Research, 113*, 52-57.

Han, J. M., Park, H. J., Kim, J. H., Jeong, D. S., & Kang, J. C. (2019). Toxic effects of arsenic on growth, hematological parameters, and plasma components of starry flounder, *Platichthys stellatus*, at two water temperature conditions. *Fisheries and Aquatic Sciences, 22*, 1-8.

Hayat, S., Javed, M., & Razzaq, S. (2007). Growth performance of metal stressed major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* reared under semi-intensive culture system. *Pakistan Veterinary Journal, 27*, 8-12.

Hussain, S. M., Javed, M., Asghar, S., Hussain, M., Abdullah, S., Raza, S. A., & Javid, A. (2010). Studies on growth performance of metals mixture stressed *Cirrhina mrigala* in earthen ponds. *Pakistan Journal of Agricultural Sciences, 47*, 263-270.

Islam, S. S., Shah, M. S. & Rahi, M. L. (2010). Study of fecundity and induced breeding of *Mystus vittatus*. *Bangladesh Journal of Zoology, 39*(2), 205-212.

Islam, S. S., Shah, M. S. & Rahi, M. L. (2014). Assessment of genetic variability of prawn (*Macrobrachium rosenbergii*) post larvae (PL) from the broods stocked under different sex ratios. *International Journal of Aquaculture, 4*(9), 10.5376.

Islam, S. S., Shah, M. S., Shams, F. I., Ali, M. R. & Rahi, M. L. (2015). Genetic variability assay of different natural and hatchery populations of Rohu (*Labeo rohita*) in Bangladesh. *International Journal of Life Sciences, 9*(1), 30-36.

Islam, S. M., Zahangir, M. M., Ashaf-Ud-Doulah, M., Khatun, M. M., & Shahjahan, M. (2020). Extreme warm acclimation temperature alters oxygen consumption, micronucleus formation in erythrocytes, and gill morphology of rohu (*Labeo rohita*) fingerlings. *Fish Physiology and Biochemistry, 46*(6), 2323-2330.

Javed, M., & Usmani, N. (2017). An overview of the adverse effects of heavy metal contamination on fish health. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 89*, 389–403.

Jezierska, B., Ługowska, K., & Witeska, M. (2009). The effects of heavy metals on embryonic development of fish (a review). *Fish Physiology and Biochemistry, 35*, 625-640.

Kokou, F., Con, P., Barki, A., Nitzan, T., Slosman, T., Mizrahi, I., & Cnaani, A. (2019). Short- and long-term low-salinity acclimation effects on the branchial and intestinal gene

expression in the European seabass (*Dicentrarchus labrax*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 231*, 11-18.

Kumari, B., Kumar, V., Sinha, A. K., Ahsan, J., Ghosh, A. K., Wang, H., & De Boeck, G. (2016). Toxicology of arsenic in fish and aquatic systems. *Environmental Chemistry Letters, 15*, 43-64.

Kundu, M., Ghosh, P., Mitra, S., Das, J., Sau, T., Banerjee, S., States, J. C., & Giri, A. K. (2011). Precancerous and non-cancer disease endpoints of chronic arsenic exposure: The level of chromosomal damage and XRCC3 T241M polymorphism. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 706*, 7-12.

Li, K., Olsen, R. E., Østensen, M. A., Altin, D., Kjørsvik, E., & Olsen, Y. (2016). Atlantic cod (*Gadus morhua*) larvae can biosynthesis phospholipid de novo from 2-oleoyl-glycerol and glycerol precursors. *Fish physiology and biochemistry, 42*(1), 137-147.

Li, M., Yu, N., Qin, J. G., Li, E., Du, Z., & Chen, L. (2014). Effects of ammonia stress, dietary linseed oil and *Edwardsiella ictaluri* challenge on juvenile dark barbel catfish *Pelteobagrus vachelli*. *Fish and Shellfish Immunology, 38*(1), 158-165.

Loughland, I., & Seebacher, F. (2020). Differences in oxidative status explain variation in thermal acclimation capacity between individual mosquito fish (*Gambusia holbrooki*). *Functional Ecology, 10*, 1-11.

Moshtaghi, A., Rahi, M. L., Mather, P. B. & Hurwood, D. A. (2017). Understanding the genomic basis of adaptive response to variable osmotic niches in freshwater prawns: a comparative intraspecific RNA-Seq analysis of *Macrobrachium australiense*. *Journal of Heredity, 108*(5), 544-552.

Moshtaghi, A., Rahi, M. L., Mather, P. B. & Hurwood, D. A. (2018). An investigation of gene expression patterns that contribute to osmoregulation in *Macrobrachium australiense*: Assessment of adaptive responses to different osmotic niches. *Gene Reports, 13*, 76-83.

Mridul, M. M. I., Zeehad, M. S. K., Aziz, D., Salin, K. R., Hurwood, D. A. & Rahi, M. L. (2024). Temperature induced biological alterations in the major carp, Rohu (*Labeo rohita*): Assessing potential effects of climate change on aquaculture production. *Aquaculture Reports, 35*, 101954.

Muhammad, S., Akram, W., Aziz, D. & Rahi, M. L. (2023). Effects of ammonia on the biological traits of the orange mud crab (*Scylla olivacea*). *Marine Reports, 2*(2), 73-94.

Nath, R. D., Rahi, M. L., Hossain, G. S. & Huq, K. A. (2008). Marketing status of freshwater snail in Khulna district. *Bangladesh Research Publication Journal, 1*(4), 337-347.

Naujokas, M. F., Anderson, B., Ahsan, H., Aposhian, H. V., Graziano, J. H., Thompson, C., & Suk, W. A. (2013). The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. *Environmental Health Perspectives, 121*(3), 295-302.

Oremland, R. S., & Stolz, J. F. (2003). The ecology of arsenic. *Science, 300*(5621), 939-944.

Pedlar, R. M., Ptashynski, M. D., Evans, R., & Klaverkamp, J. F. (2002). Toxicological effects of dietary arsenic exposure in Lake Whitefish (*Coregonus clupeaformis*). *Aquatic Toxicology, 57*, 167-189.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research, 29*(9), e45–e45.

Rahi, M. L. & Shah, M. S. (2012). Triploidization in rohu× mrigal hybrid and comparison of growth performance of triploid hybrid. *Aquaculture Research, 43*(12), 1867-1879.

Rahi, M. L. (2017). *Understanding the molecular basis of adaptation to freshwater environments by prawns in the genus Macrobrachium* [Doctoral dissertation]. Queensland University of Technology. https://doi.org/10.5204/thesis.eprints.118051.

Rahi, M. L., Amin, S., Mather, P. B. & Hurwood, D. A. (2017). Candidate genes that have facilitated freshwater adaptation by palaemonid prawns in the genus *Macrobrachium*:

identification and expression validation in a model species (*M. koombooloomba*). *PeerJ, 5*, e2977.

Rahi, M. L., Moshtaghi, A., Mather, P. B. & Hurwood, D. A. (2018). Osmoregulation in decapod crustaceans: physiological and genomic perspectives. *Hydrobiologia, 825*, 177-188.

Rahi, M. L., Mather, P. B., Ezaz, T. & Hurwood, D. A. (2019). The molecular basis of freshwater adaptation in prawns: Insights from comparative transcriptomics of three *Macrobrachium* species. *Genome Biology and Evolution, 11*(4), 1002-1018.

Rahi, M. L., Ferdusy, T., Ahmed, S. W., Khan, M. N., Aziz, D., & Salin, K. R. (2020). Impact of salinity changes on growth, oxygen consumption and expression pattern of selected candidate genes in the orange mud crab (*Scylla olivacea*). *Aquaculture Research, 51*(10), 4290–4301.

Rahi, M.L., Mather, P.B., & Hurwood, D.A. (2021). Do plasticity in gene expression and physiological responses in Palaemonid prawns facilitate adaptive response to different osmotic challenges? *Comparative Biochemistry and Physiology A, 251*, 110810.

Rahi, M. L., Mahmud, S., Dilruba, K. J., Sabbir, W., Aziz, D. & Hurwood, D. A. (2021). Temperature induced changes in physiological traits and expression of selected candidate genes in black tiger shrimp (*Penaeus monodon*) larvae. *Aquaculture Reports, 19*, 100620.

Rahi, M. L., Azad, K. N., Tabassum, M., Irin, H. H., Hossain, K. S., Aziz, D., Moshtaghi, A. & Hurwood, D. A. (2021). Effects of salinity on physiological, biochemical and gene expression parameters of black tiger shrimp (*Penaeus monodon*): Potential for farming in low-salinity environments. *Biology, 10*, 1220.

Rahi, M. L., Sabbir, W., Salin, K. R., Aziz, D., & Hurwood, D. A. (2022). Physiological, biochemical and genetic responses of black tiger shrimp (*Penaeus monodon*) to differential exposure to white spot syndrome virus and *Vibrio parahaemolyticus*. *Aquaculture, 546*, 737337.

Rahman, M. H., Haque, M. M., Alam, M. A., & Rahman, F. (2022). A study on the specific growth rate (SGR) at different stages of Tilapia (*Oreochromis niloticus*) production cycle in tank based aquaculture system. *International Journal of Aquaculture and Fishery Sciences, 8*(2), 59–65.

Rahi, M. L., Mather, P. B., de Bello Cioffi, M., Ezaz, T. & Rahi, M. L. (2023). Genomic basis of freshwater adaptation in the Palaemonid prawn genus *Macrobrachium*: convergent evolution following multiple independent colonization events. *Journal of Molecular Evolution, 91*, 976–989.

Rajendiran, S., Iqbal, B. M., & Vasudevan, S. (2016). Induced thermal stress on serotonin levels in the blue swimmer crab, *Portunus pelagicus*. *Biochemistry and Biophysics Reports, 5*, 425-429.

Randall, D. J. (1990). Oxygen, carbon dioxide and ammonia transfer across teleost fish gills. In *Fish Physiology, Fish Toxicology, and Fisheries Management: Proceedings of an International Symposium, Guangzhou, PRC, September 14-16, 1988* (p. 135). Environmental Research Laboratory, Office of Research and Development, US Environmental Protection Agency.

Ratnaike, R. N. (2003). Acute and chronic arsenic toxicity. *Postgraduate Medical Journal, 79*(933), 391-396.

Rogl, K. A., Rahi, M. L., Royle, J., Prentis, P. J. & Hurwood, D. A. (2018). A transcriptome-wide assessment of differentially expressed genes among two highly divergent, yet sympatric, lineages of the freshwater Atyid shrimp, *Paratya australiensis*. *Hydrobiologia, 825*(1), 189-196.

Rosas, C., Cuzon, G., Gaxiola, G., Le Priol, Y., Pascual, C., Rossignyol, J., Contreras, F., Sanchez, A., & Van Wormhoudt, A. (2001). Metabolism and growth of juveniles of

Litopenaeus vannamei: effect of salinity and dietary carbohydrate levels. *Journal of Experimental Marine Biology and Ecology, 259*(1), 1-22.

Sabbir, W., Masud, M. A. A., Islam, S. S., Rahman, M. A. & Rahi, M. L. (2010). Some aspects of water quality parameters of the Mouri River, Khulna: An attempt to estimate pollution status. *Bangladesh Research Publication Journal, 4*(1), 95-102.

Sabbir, W., Khan, M. N., Sultana, S., Rahi, M. L. & Shah, M. S. (2017). Production of heterotic hybrid in Rohu (*Labeo rohita*) by crossing the riverine and hatchery strains. *International Journal of Innovation Sciences and Research, 6*(2), 982-986.

Sahu, D. K., Panda, S. P., Meher, P. K., Das, P., Routray, P., Sundaray, J. K., Jayasankar, P., & Nandini, S. (2015). Construction, de-novo assembly and analysis of transcriptome for identification of reproduction-related genes and pathways from Rohu, *Labeo rohita* (Hamilton). *PLOS One, 10*(7), e0132450.

Selvi, R. T., & Ilavazhahan, M. (2015). Histopathological changes in gill tissue of the fish *Catla catla* exposed to sublethal concentration of pesticide methyl parathion and a heavy metal ferous sulphate. *Biomedical and Pharmacology Journal, 5*(2), 305-312.

Shah, M. S., Ghosh, A. K., Rahi, M. L., Huq, K. A., Rahaman, S. M. B. & Sabbir, W. (2011). Production of heterotic hybrid in rohu (*Labeo rohita*) through strain crossing. *International Journal of Life Sciences, 5*(1), 32-38.

Sinha, A. K., Liew, H. J., Diricx, M., Kumar, V., Darras, V. M., Blust, R., & De Boeck, G. (2012). Combined effects of high environmental ammonia, starvation and exercise on hormonal and ion-regulatory response in goldfish (*Carassius auratus*). *Aquatic Toxicology, 114*, 153-164.

Triantaphyllopoulos, K. A., Cartas, D., & Miliou, H. (2020). Factors influencing GH and IGF-I gene expression on growth in teleost fish: how can aquaculture industry benefit? *Reviews in Aquaculture, 12*(3), 1637-1662.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—new capabilities and interfaces. *Nucleic Acids Research, 40*(15), e115-e115.

Vutukuru, S. S., Arun Prabhath, N., Raghavender, M., & Yerramilli, A. (2007). Effect of arsenic and chromium on the serum amino-transferases activity in Indian major carp, *Labeo rohita. International Journal of Environmental Research and Public Health, 4*(3), 224-227.

Wedemeyer, G. (1996). *Physiology of fish in intensive culture systems*. Springer Science & Business Media.

Witeska, M., Kondera, E., Ługowska, K., & Bojarski, B. (2022). Hematological methods in fish–Not only for beginners. *Aquaculture, 547*, 737498.

Zarantoniello, M., Bortoletti, M., Olivotto, I., Ratti, S., Poltronieri, C., Negrato, E., & Bertotto, D. (2021). Salinity, temperature and ammonia acute stress response in sea bream (Sparus aurata) juveniles: A multidisciplinary study. *Animals, 11*(1), 97.

Zeehad, M. S. K., Mridul, M. M. I., Chakrobortty, D., Mahfuj, S., Aziz, D., Hurwood, D. A. & Rahi, M. L. (2024). Effects of ammonia on the cellular, physiological, biochemical and genetic traits of Indian major carp (*Labeo rohita*) fry in artisanal Bangladeshi aquaculture. *Aquaculture Fish and Fisheries, 4*(1), e160.

Zeynali, M., Nafisi B. M., Morshedi, V., Ghasemi, A., & Torfi M. M. (2020). Replacement of dietary fishmeal with *Sargassum ilicifolium* meal on growth, innate immunity and immune gene mRNA transcript abundance in *Lates calcarifer* juveniles. *Aquaculture Nutrition, 26*, 1657-1668.