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# Physiological and Haematological Stress Responses of *Labeo catla* Fingerlings in Relation to Stocking Density during Pre-transport Conditioning

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## ABSTRACT

The study was conducted from August–September, 2024 at the Fish Seed Production Center and Fish Farm of the College of Fisheries Science, Nagpur, Maharashtra, India to systematically evaluate metabolic and stress-related responses of *Labeo catla* fingerlings under varying stocking densities to determine and standardize the optimal conditioning density that minimizes stress, reduces mortality, and enhances post-transport efficiency. In this study, Catla fingerlings (90.82±5.91 mm) were conditioned in a flow-through system with a water exchange rate of 6.5 l $min^{-1}$ for the period of 24 h, during this, fingerlings were stocked at four densities: 1 fingerling $l^{-1}$, 2 fingerlings $l^{-1}$, 3 fingerlings $l^{-1}$, and 4 fingerlings $l^{-1}$. During conditioning, blood samples were collected for glucose, lactate, hemoglobin, and hematocrit estimation, while muscle samples were used for glycogen analysis following anesthesia with clove oil and humane euthanasia. Water quality parameters such as dissolved oxygen and total ammonia nitrogen were monitored to assess metabolic load under varying densities. The results showed density-dependent stress responses. 4 fingerlings $l^{-1}$ exhibited significantly higher blood glucose and lactate, lower glycogen levels, and greater mortality ($p$<0.05), indicating elevated stress. While water quality remained stable overall, 4 fingerlings $l^{-1}$ showed more DO depletion and total ammonia buildup. 3 fingerlings $l^{-1}$ showed the best balance between stocking pressure and physiological stability, indicating it was the optimal conditioning density for Catla fingerlings in flow-through systems.

**KEYWORDS:** *Labeo catla*, stress physiology, haematology, pre-transport conditioning

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***Data Availability Statement:*** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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## 1. INTRODUCTION

Catla (*Labeo catla*), known for its rapid growth and economic value, recorded a production of over 4 mt in 2022 and is the only Indian fish species to rank 8th among the world's top 10 aquaculture species (Anonymous, 2024), highlighting its vital role in both national and global freshwater aquaculture. Fish fingerling transportation is a vital yet often overlooked component of aquaculture, influencing disease control and overall productivity (Husen et al., 2021). Efficient movement of fish seed, particularly fingerlings, from hatcheries to grow-out systems is essential for sustaining aquaculture, linking seed production to farming operations (Putri et al., 2020; Shah, 2021). Despite advancements in aquaculture technology, pre-transport stress management remains a critical challenge for ensuring fish survival and quality post-transport (Bais, 2018). Handling, stocking density, and environmental conditions play crucial roles during conditioning and transportation processes (Nair et al., 2023; Sutphin and Hueth, 2015). Stocking density is a major determinant of stress in aquaculture systems as it directly affects oxygen availability, metabolic rates, and fish health (Dediu et al., 2021; Swain et al., 2022; Klug et al., 2021; Suguna, 2020). High stocking densities increase fish-to-fish interactions, reduce space availability, intensify competition for oxygen and feed resources, and trigger physiological stress responses (Tan et al., 2018; Swain et al., 2022; Nair et al., 2023). Elevated stress induces cortisol secretion, which subsequently leads to hyperglycemia, increased lactate levels, and depletion of glycogen reserves, resulting in impaired immune function, reduced growth, and higher mortality rates (Bi et al., 2023; Schreck et al., 2001). Although the beneficial effects of maintaining optimal stocking densities are well established for many fish species, limited information is available on the influence of stocking density during the conditioning phase of *Labeo catla* fingerlings. Pre-transport conditioning refers to the acclimation process in which fish are held under controlled conditions prior to transportation to reduce metabolic activity, evacuate gut contents, and enhance resilience to handling and transport stress. It is an essential strategy aimed at minimizing stress and mortality during transport (Schreck et al., 1995; Lim et al., 2003; Nair et al., 2023; Conte, 2004). This process, known as pre-transport conditioning, involves acclimating fish to specific environmental conditions such as water quality, temperature, and reduced feeding, thereby decreasing their sensitivity to subsequent handling and transportation (Kamalam et al., 2017). During conditioning, variations in stocking density influence stress indicators like blood glucose, lactate, hemoglobin, and hematocrit, revealing the physiological adaptability of fish. Understanding these responses in *Labeo catla* fingerlings supports the development of strategies to reduce stress and improve post-transport survival (Husen and Sharma, 2014; Zhang et al., 2023; Minahal et al., 2024). Glucose and lactate levels increase in response to acute stress, indicating increased energy mobilization and anaerobic metabolism (Thomas and Robertson, 1991). Similarly, hemoglobin and hematocrit values fluctuate in response to oxygen transport demands, reflecting metabolic adaptation under varying stocking densities (Davison et al., 2023). Gut evacuation is a critical physiological process during pre-transport conditioning, as incomplete gut clearance can elevate metabolic waste production and ammonia accumulation, leading to reduced water quality and survival during transport (Nair et al., 2023; Conte, 2004). Monitoring gut evacuation time indicates fish readiness for transport and tolerance to stress. This study examined how stocking density affects the physiology of *Labeo catla* fingerlings during a 24 h conditioning period by analyzing metabolic indicators (blood glucose, lactate, muscle glycogen) and haematological responses (hemoglobin, hematocrit). The findings aimed to guide optimal conditioning density, minimize stress-related mortality, and enhance transport efficiency.

## 2. MATERIALS AND METHODS

### 2.1. *Study site and source of fish fingerlings*

The study was conducted from August–September, 2024 at the Fish Seed Production Center and Fish Farm of the College of Fisheries Science, Nagpur (21°09'11.6"N, 79°02'57.1"E), Maharashtra, India. The fry of Catla (measuring 40–60 mm) were procured from a commercial hatchery and seed rearing farm, Mandhare Seed Production and Rearing Farm, Kuhi-Mandal, Nagpur, India. Upon arrival, the fry were stocked in an earthen nursery pond at a density of 20–30 fry $m^{-2}$ (Ayyappan et al., 2011) and reared under semi-intensive conditions. Fish were fed a diet containing 35% crude protein. These fry attained an average size of 80–100 mm (fingerling stage) after approximately 30 days of rearing. Following 30 days of rearing, the fingerlings were netted and collected for the pre-transport conditioning experiment

### 2.2. *Experimental setup*

The experiment was conducted using 20 circular tanks (1000 l capacity each) operated under a flow-through system with a water exchange rate of 6.5 l $min^{-1}$. Each tank maintained an effective water volume of 500 l. Fish were conditioned at four stocking densities 1, 2, 3, and 4 fingerlings $l^{-1}$ ($T_1$, $T_2$, $T_3$, and $T_4$) with five replicate tanks per treatment. Accordingly, each tank contained 500, 1000, 1500, and 2000 fingerlings, respectively. The conditioning period lasted for 24 h, during which water quality parameters were continuously monitored.

*2.3. Data collection*

Physiological stress indicators, including blood glucose, lactate, glycogen levels, hemoglobin (Hb), and hematocrit (HcT), were measured at 1 h, 4 h, 8 h, 12 h, 16 h, 20 h, and 24 h of conditioning.

*2.4. Gut emptying time and assessment of weak, isolated, and dead fingerlings*

Fish behavior and mortality were observed every 4 h by trained personnel working in shifts. Weak fish were identified based on lethargy (reduced activity), diminished response to gentle tactile stimuli, and irregular swimming patterns. Gut evacuation time was assessed from the presence or absence of fecal matter in the water. Mortality was recorded at each interval, and dead fish were promptly removed to maintain water quality.

*2.5. Physico-chemistry of water*

Prior to initiating the experiment, baseline water quality parameters including temperature, pH, dissolved oxygen (DO), total alkalinity, total hardness, total ammonia nitrogen (TAN), and free carbon dioxide ($CO_2$) were measured to confirm uniform water quality across all tanks. Throughout the conditioning period, these parameters were continuously monitored at 4 h intervals to ensure they remained within optimal ranges. These involved measuring water temperature (Multi-Stem Thermometer); pH (Thermo Scientific digital pH meter); dissolved oxygen (DO) (Aquasol-AM-DO-01); total alkalinity (Hanna Instrument-HI775); total ammonia nitrogen (TAN) (analyzed spectrophotometrically); free carbon dioxide ($CO_2$) and total hardness were analysed titrimetrically following Anonymous (2005).

*2.6. Collection, preparation, and estimation of samples*

Prior to blood collection, fish were anesthetized using clove oil (50 µl $l^{-1}$). Blood samples were obtained via caudal venipuncture using heparinized 1 ml syringes and transferred into EDTA-coated microtubes. Approximately 100–120 µl of blood was collected from fingerling, which was sufficient for the estimation of glucose, lactate, hemoglobin, and hematocrit levels.

Blood glucose levels were measured using a OneTouch Ultra-Glucose Meter (Bartonkova et al., 2016). The hemoglobin concentration was determined using the cyanomethemoglobin method with Drabkin's reagent (Qualigen). In this method, 20 µl of blood was mixed with Drabkin's working solution in a test tube, and the absorbance was recorded at 540 nm using a UV-VIS spectrophotometer. The final hemoglobin concentration was calculated by comparing the absorbance values with those of a standard cyanomethemoglobin solution (Qualigen Diagnostic).

Glycogen estimation was done from fish muscles. For this, the fish were anesthetized with clove oil and euthanized by decapitation. Muscle tissue samples were dissolved in 30% potassium hydroxide (KOH) and further treated with 95% ethanol. Glycogen content was estimated using an enzymatic colorimetric method (Hassid and Abraham, 1957) with anthrone reagent.

Hematocrit was determined by centrifugation of heparinized capillary tubes containing approximately 65–70 µl of blood collected from fingerling. Blood lactate levels were measured using a commercially available portable lactate meter (Lacto-spark) following standard methodologies (Wells and Pankhurst, 1999; Brown et al., 2008; Vaage et al., 2023).

*2.7. Statistical analysis*

Percentage data for survival, weak, isolated, and cumulative mortality were obtained and arcsine transformed to stabilize variance and normalize distribution. Transformed data were analyzed using one-way ANOVA followed by Tukey's HSD post-hoc test to identify significant differences ($p$<0.05). Statistical analyses were conducted using SPSS software (version 16.0).

## 3. RESULTS AND DISCUSSION

*3.1. Gut evacuation time and survival during conditioning*

As the stocking density increased from 1 to 4 fish $l^{-1}$, the gut evacuation time declined logarithmically (Figure 1), with a strong relationship ($R^2$=0.9848) indicating that 98.48% of the variation in gut evacuation time was explained by changes in stocking density. This suggested that at higher densities, intensified competition and limited individual space accelerate food passage, potentially reducing the digestion efficiency (Jia et al., 2021). This outcome might be attributed to the crowding stress experienced by fish under higher stocking densities, which likely triggered physiological responses such as elevated metabolic activity and enhanced gut motility (Dediu et al., 2021; Alvarez et al.,

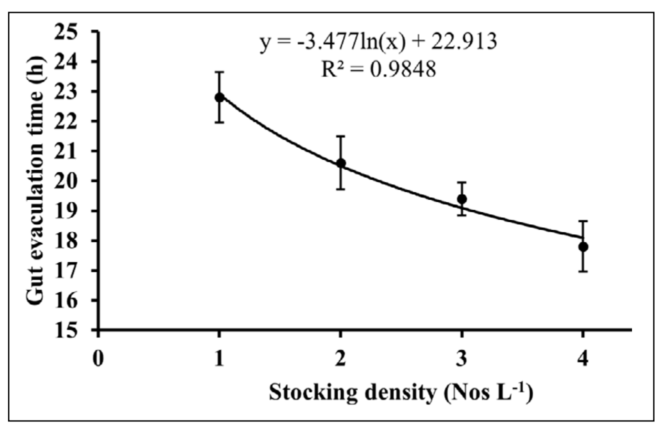


Figure 1: The gut evacuation time recorded in *Labeo catla* fingerlings stocked at different densities during conditioning

2022). In our observations, fish appeared more restless and active in high-density treatments, indicating stress-induced behavioral changes, leading to faster gut clearance as fish diverted energy from digestion toward immediate survival mechanisms (Paredes et al., 2020). This physiological shift under stress conditions aligned with the pattern observed in our study.

Survival recorded at the time of gut evacuation and 24 h post-conditioning (Figure 2) indicated that $T_1$, $T_2$, and $T_3$ had comparable survival rates, whereas $T_4$ showed significantly lower survival at both time points. This reduction in survival might be attributed to increased crowding stress at higher stocking densities, which could elevate metabolic activity, raise oxygen demand, and lead to greater waste accumulation (Dawood et al., 2022; Carbonara et al., 2023). This suggested that higher densities imposed physiological stress, leading to increased mortality. The decline in survival in $T_4$ at 24 h highlighted the prolonged stress effects, emphasizing the necessity of optimized stocking densities in pre-transportation conditioning practice to balance production efficiency and fish welfare (Portz et al., 2006).

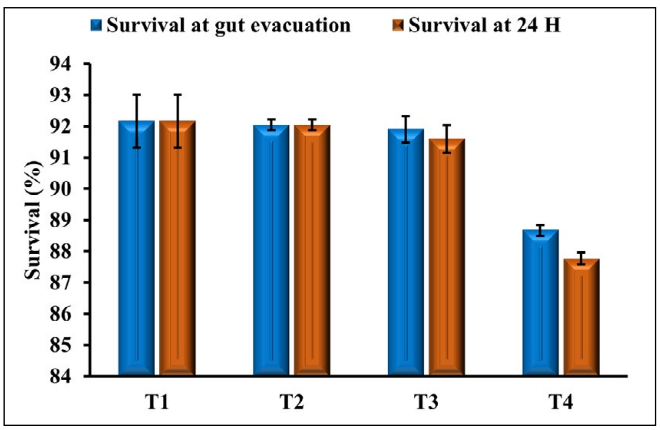


Figure 2: Survival of *Labeo catla* fingerlings after gut evacuation and 24 hours conditioning at different densities: $T_1$=1 fish l$^{-1}$, $T_2$=2 fish l$^{-1}$, $T_3$=3 fish l$^{-1}$, and $T_4$=4 fish l$^{-1}$. Values expressed as mean±S.D

*3.2. Secondary stress responses during conditioning*

*3.2.1. Blood glucose and lactate dynamics*

The stocking density significantly influenced the blood glucose levels during conditioning (Figure 3). $T_4$ exhibited the highest glucose levels, indicating elevated stress, whereas $T_1$ maintained the lowest levels, suggesting minimal stress. Over time, glucose levels declined across all the treatments, but $T_4$ remained significantly higher at 24 h, suggesting incomplete recovery. This response could be attributed to the physiological activation of the hypothalamic-pituitary-interrenal (HPI) axis under crowding stress, leading to cortisol release that stimulates gluconeogenesis and raises

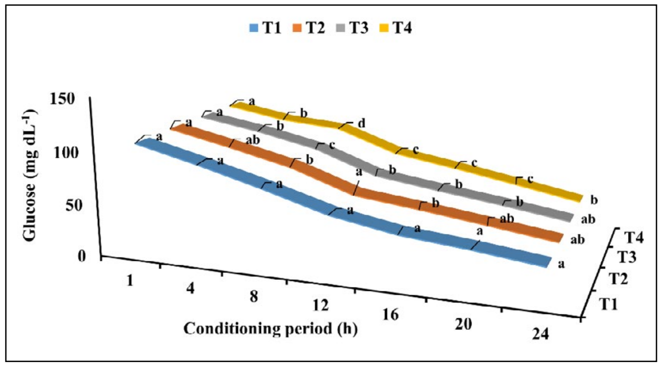


Figure 3: The blood glucose levels during a 24 h conditioning period in *Labeo catla* fingerlings, stocked at different densities: $T_1$=1 fish l$^{-1}$, $T_2$=2 fish l$^{-1}$, $T_3$=3 fish l$^{-1}$, and $T_4$=4 fish l$^{-1}$. Values are expressed as mean±S.D. Values with different superscripts indicate significant differences among treatments ($p \le 0.05$), while means sharing the same letter, do not differ significantly ($p > 0.05$)

blood glucose (Pickering et al., 1989; Barton et al., 1991; Mommsen et al., 1999). The higher the density, the more intense the stress response, resulting in prolonged hyperglycemia. This aligned with the findings on *Labeo rohita* and *Oreochromis niloticus* (Barcellos et al., 2001; Ruane et al., 2002), in which higher stocking densities led to prolonged stress-induced hyperglycemia.

Blood lactate levels followed a similar trend (Figure 4), initially spiking at 1 h across all treatments, likely due to acute stress from handling and stocking (Hoseini et al., 2024). This rise probably resulted from a shift to anaerobic metabolism triggered by crowding-induced oxygen limitation, leading to increased glycolysis and lactate accumulation (Barton et al., 1991; Ruane et al., 2002; Sousa et al., 2021). Although lactate levels declined after 4 and 8 h, $T_4$ remained significantly higher, indicating

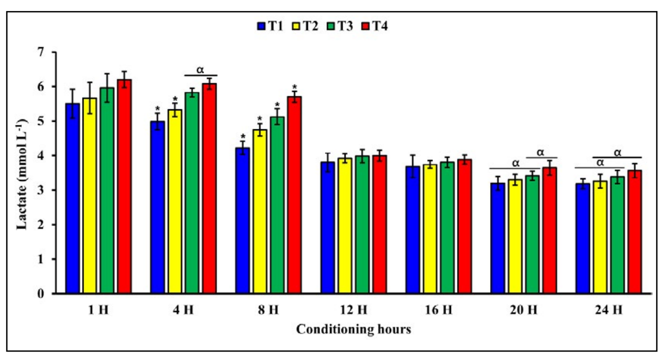


Figure 4: The blood lactate levels during a 24 h conditioning period in *Labeo catla* fingerlings, stocked at different densities: $T_1$=1 fish l$^{-1}$, $T_2$=2 fish l$^{-1}$, $T_3$=3 fish l$^{-1}$, and $T_4$=4 fish l$^{-1}$. Values were mean±S.D. α denoted non-significance ($p > 0.05$) among treatments; groups not sharing α differ significantly ($p < 0.05$). Absence of symbol indicated no difference; * denoted significant variation from other treatments

delayed recovery. Sustained elevations at 20 and 24 h in $T_4$ confirm prolonged metabolic stress. Similar behavior has been observed in *Cyprinus carpio* and *Pagrus pagrus*, where higher stocking densities necessitated greater reliance on anaerobic metabolism (Hematyar et al., 2024; Dobsikova et al., 2009).

*3.2.2. Glycogen utilization and energy metabolism*

Initially glycogen levels declined across all the treatments, reflecting stress-induced energy depletion, before increasing over the 24 h period (Figure 5). However, $T_4$ exhibited significantly lower glycogen levels during 4–20 h, indicating higher energy expenditure to counteract the stress ((Liu et al., 2016; Faudzi et al., 2021). Studies on Indian major carps and *Pagrus pagrus* have yielded similar outcome (Laiz-Carrion et al., 2012; Chatterjee et al., 2006). High stocking densities were seen to accelerated glycogen depletion owing to increased metabolic demands.

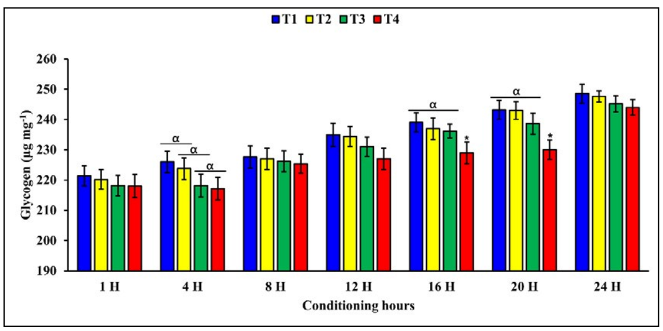


Figure 5: The glycogen levels during a 24 h conditioning period in *Labeo catla* fingerlings, stocked at different densities: $T_1$: 1 fingerling $l^{-1}$, $T_2$: 2 fingerlings $l^{-1}$, $T_3$: 3 fingerlings $l^{-1}$, and $T_4$: 4 fingerlings $l^{-1}$. Values were mean±S.D. α denotes non-significance ($p$>0.05) among treatments; groups not sharing α differ significantly ($p$<0.05). Absence of symbol indicated no difference; *Denoted significant variation from other treatments

In this case, metabolic stress has been reported to causes rapid energy expenditure, leading to glycogen breakdown as fish attempt to sustain homeostasis (Polakof et al., 2012). Fish exposed to high stocking densities exhibit increased reliance on glycolysis, depleting glycogen stores at a faster rate (Dai et al., 2024). The depletion of glycogen reserves in $T_4$ suggested a prolonged stress response, in which fish were forced to mobilize more of the stored energy to meet metabolic demands.

*3.2.3. Haematological responses*

Haematological parameters remained relatively stable at the four stocking densities (Table 1). Hemoglobin (Hb) and hematocrit (HcT) levels exhibited a gradual decline over 24 h with no significant differences among treatments ($p$>0.05). This stability might be attributed to the ability of *Labeo catla* fingerlings to manage acute stress during short-term (≤24 h) exposure without substantial disruption of their oxygen transport capacity; however, significant haematological disruption was generally observed under prolonged or chronic stress beyond 48–72 h (Pakhira et al., 2015; Li et al., 2024; Barton and Iwama, 1991). Despite the crowding-induced stress, the physiological resilience of the fish appeared sufficient to maintain stable haematological parameters over the conditioning period. This suggested that *Labeo catla* has special adaptive mechanisms to maintain oxygen capacity under varying densities. Studies on *Labeo rohita* and *Cyprinus carpio* have reported similar trends, where stocking density did not significantly alter

Table 1: Effect of stocking density on hemoglobin (Hb) and hematocrit (HcT) levels in *Labeo catla* fingerlings during a 24 h conditioning period

| Parameter | Period after stocking (h) | Stocking density | | | |
|---|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| Hb (g $dl^{-1}$) | 1 | 9.56±0.30 | 9.60±0.29 | 9.72±0.25 | 9.76±0.27 |
| | 4 | 9.32±0.52 | 9.44±0.65 | 9.58±0.44 | 9.70±0.39 |
| | 8 | 9.26±0.46 | 9.36±0.69 | 9.54±0.40 | 9.60±0.57 |
| | 12 | 9.10±0.25 | 9.32±0.30 | 9.42±0.39 | 9.54±0.38 |
| | 16 | 9.02±0.42 | 9.10±0.26 | 9.18±0.56 | 9.32±0.41 |
| | 20 | 8.84±0.43 | 8.94±0.22 | 9.06±0.51 | 9.24±0.23 |
| | 24 | 8.40±0.41 | 8.58±0.19 | 8.66±0.21 | 9.00±0.46 |
| HcT (%) | 1 | 28.60±0.89 | 28.80±1.79 | 29.00±1.41 | 29.80±2.39 |
| | 4 | 28.00±2.24 | 28.40±0.55 | 28.80±1.92 | 29.40±1.14 |
| | 8 | 27.20±2.28 | 27.60±1.14 | 28.40±1.82 | 29.00±0.71 |
| | 12 | 27.00±1.58 | 27.20±1.64 | 28.20±0.84 | 28.60±1.67 |
| | 16 | 26.80±1.10 | 27.00±1.00 | 28.00±1.22 | 28.20±1.92 |
| | 20 | 25.40±2.19 | 26.80±0.84 | 27.40±2.07 | 27.80±1.64 |
| | 24 | 24.40±0.89 | 25.00±1.58 | 25.40±0.89 | 26.40±1.67 |

Values were expressed as the mean±S.D.; Stocking densities: $T_1$: 1 fingerling $l^{-1}$; $T_2$: 2 fingerlings $l^{-1}$; $T_3$: 3 fingerlings $l^{-1}$; $T_4$: 4 fingerlings $l^{-1}$

haematological parameters, likely because of species-specific physiological adaptations (Pakhira et al., 2015; Dobsikova et al., 2009).

### 3.3. Survival and stress-induced mortality

Stocking density during conditioning strongly influenced mortality, with higher densities correlating with increased mortality (Table 2). While no mortality was recorded at 1 h, $T_3$ and $T_4$ showed a significantly higher % of weak, isolated, and dead fish from 4 to 12 h, indicating stress effects under overcrowded conditions. The cumulative mortality remained highest in $T_4$ at 24 h, likely due to increased competition for oxygen and space (Habib et al., 2022; Swain et al., 2022), resulting in metabolic exhaustion and reduced recovery ability. Comparable outcomes have been reported in *Labeo rohita* and *Colossoma macropomum*, where elevated stocking densities intensified stress-induced mortality through oxygen depletion and metabolic overload (Islam and Hossain, 2013; Gomes et al., 2003).

Table 2: Proportion of weak, isolated, and cumulatively dead *Labeo catla* fingerlings at different stocking densities over a 24 h conditioning period

| Period after stocking (h) | Weak fish (%) | | | | Isolated fish (%) | | | | Cumulatively dead fish (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| 1 | 0.24±0.09[a] | 0.24±0.05[a] | 0.25±0.06[a] | 0.43±0.06[b] | 0.24±0.09 | 0.24±0.05 | 0.25±0.06 | 0.28±0.07 | NIL | NIL | NIL | NIL |
| 4 | 1.92±0.23[a] | 1.96±0.17[a] | 1.97±0.12[a] | 2.29±0.14[b] | 0.92±0.30 | 0.96±0.11 | 0.99±0.10 | 1.03±0.04 | 1.16±0.17[a] | 1.88±0.44[b] | 1.60±0.11[b] | 2.02±0.12[b] |
| 8 | 1.38±0.33[a] | 1.51±0.22[a] | 1.94±0.09[b] | 2.06±0.14[b] | 0.89±0.23 | 0.90±0.08 | 0.93±0.09 | 0.95±0.10 | 2.80±0.32[a] | 3.82±0.33[b] | 3.35±0.14[c] | 4.67±0.15[d] |
| 12 | 1.48±0.18[a] | 1.52±0.12[a] | 1.59±0.11[a] | 2.43±0.09[b] | 0.58±0.17 | 0.58±0.12 | 0.58±0.08 | 0.61±0.06 | 4.60±0.47[a] | 5.00±0.31[ab] | 5.24±0.18[b] | 7.32±0.23[c] |
| 16 | 1.22±0.18[a] | 1.28±0.20[a] | 1.42±0.11[bc] | 1.58±0.09[c] | 0.38±0.09 | 0.38±0.06 | 0.38±0.04 | 0.41±0.10 | 6.36±0.71[a] | 6.36±0.22[a] | 6.85±0.32[a] | 9.59±0.18[b] |
| 20 | 0.60±0.18[a] | 0.62±0.09[a] | 0.66±0.10[a] | 1.01±0.06[b] | 0.26±0.18 | 0.26±0.16 | 0.21±0.07 | 0.29±0.07 | 7.32±0.76[a] | 7.50±0.16[a] | 8.09±0.41[a] | 11.32±0.16[b] |
| 24 | 0.39±0.10 | 0.39±0.06 | 0.41±0.04 | 0.42±0.06 | 0.26±0.18 | 0.22±0.08 | 0.22±0.07 | 0.23±0.06 | 7.84±0.83[a] | 7.96±0.17[a] | 8.40±0.44[a] | 12.23±0.19[b] |

Values were expressed as the mean±S.D. Values in a row with different superscripts for each factor indicate significant differences among treatments ($p$≤0.05); Stocking densities: $T_1$: 1 fingerling $l^{-1}$; $T_2$: 2 fingerlings $l^{-1}$; $T_3$: 3 fingerlings $l^{-1}$; $T_4$: 4 fingerlings $l^{-1}$

### 3.4. Water quality and its influence on stress physiology

Water quality parameters remained stable across all treatments, likely due to the flow-through system minimizing fluctuations (Fiordelmondo et al., 2020), except for dissolved oxygen (DO) and total ammonia nitrogen (TAN), which showed significant variations (Figure 6). In $T_4$, the lowest DO (5.78±0.10 mg $l^{-1}$) and the highest TAN concentration (0.04±0.01 mg $l^{-1}$) were recorded at 24 h. The decline in DO could be attributed to the higher cumulative oxygen demand from increased biomass, while elevated TAN levels reflected greater metabolic waste production at higher stocking densities (Al-Harbi et al., 2000). Previous studies on *Labeo rohita* have also shown that intensified stocking densities could aggravate oxygen depletion and ammonia accumulation, intensifying physiological stress (Pakhira et al., 2015).

Stocking density was seen to increase lactate levels indicating higher stress, while dissolved oxygen declined slightly due to increased consumption (Figure 7). This outcome could be explained by the fact that higher stocking densities lead to increased metabolic activity and crowding stress among fish, resulting in greater oxygen demand. As oxygen availability became relatively limited under such conditions, fish shift more towards anaerobic metabolism, causing an accumulation of lactate in the blood (Ardiansyah and Fotedar, 2016). Thus, elevated lactate levels reflected the physiological adjustment to hypoxic stress induced by higher biomass density in the system. Similar trends have been reported by Jia et al. (2022) and Chen et al. (2023), who studied the effects of rearing density on *Micropterus salmoides* and *Carassius auratus*, respectively.

Regression analysis (Figure 8) revealed a strong negative correlation ($R^2$=0.9606) between DO and lactate levels, indicating that reduced oxygen availability triggers anaerobic

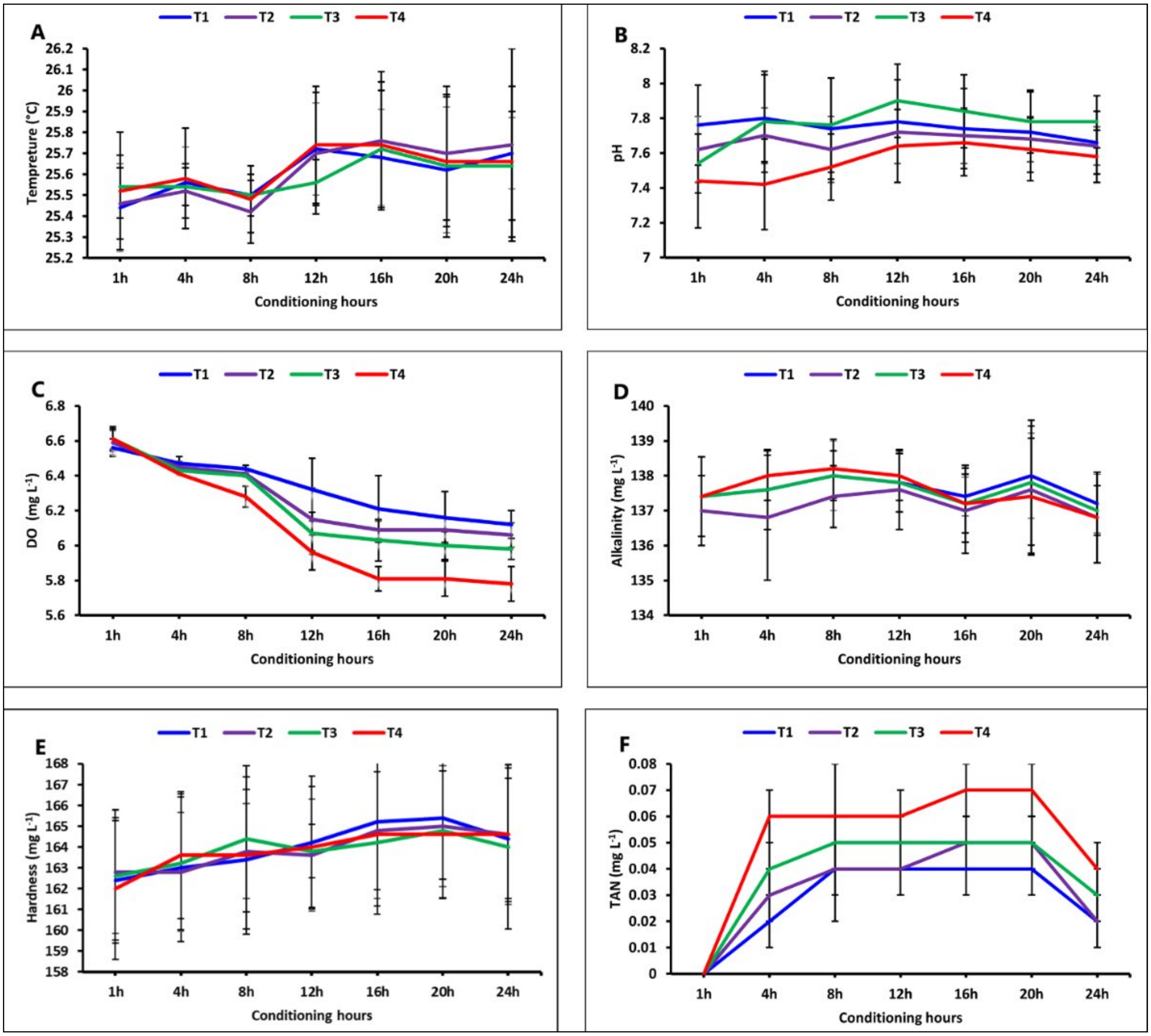


Figure 6: Water quality parameters during conditioning of Catla fingerlings up to 24 h at different stocking densities. Fish were stocked at four densities: $T_1$ (1 fingerling $l^{-1}$); $T_2$: 2 fingerlings $l^{-1}$; $T_3$: 3 fingerlings $l^{-1}$; $T_4$: 4 fingerlings $l^{-1}$. Data are expressed as mean±S.D

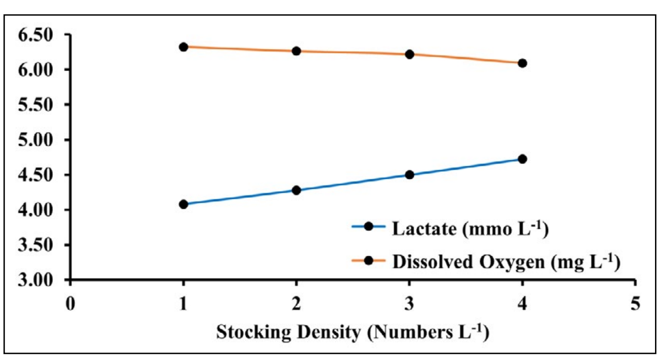


Figure 7: Effect of stocking density on lactate levels and dissolved oxygen in *Labeo catla* during a 24 h Conditioning Period

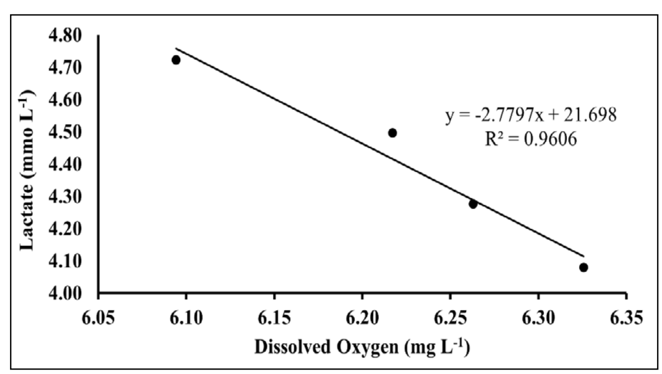


Figure 8: Regression of lactate in *Labeo catla* on dissolved oxygen levels during a 24 h conditioning

metabolism, leading to lactate accumulation. This shift towards anaerobic respiration under hypoxia has been well documented in fish (Cox, 1974; Ruiz et al., 2024). These results reinforced the importance of maintaining adequate DO levels to minimize stress and optimize fish health during pre-transport conditioning.

## 4. CONCLUSION

Stocking density strongly influenced stress physiology and survival of *Labeo catla* fingerlings during pre-transport conditioning in a flow-through system. Higher densities caused greater metabolic stress and mortality, while lower densities underutilized system capacity. $T_3$ (3 fish $l^{-1}$) proved optimal, showing stable glucose, lactate, glycogen, and haematological values with minimal DO depletion and TAN accumulation. Thus, $T_3$ ensured efficient conditioning and readiness for transport, supporting sustainable aquaculture practices.

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## 6. REFERENCES

Al-Harbi, A.H., Siddiqui, A.Q., 2000. Effects of tilapia stocking densities on fish growth and water quality in tanks. Asian Fisheries Science 13(4), 391–396. https://doi.org/10.33997/j.afs.2000.13.4.011.

Alvarez, C.A., Santana, P.A., Carcamo, C.B., Cardenas, C., Byron, M., Ramirez, F., Valenzuela, C., Boltana, S., Alcaíno, J., Guzman, F., Mercado, L., 2022. Effect of fish stock density on hormone genes expression from brain and gastrointestinal tract of *Salmo salar*. Animals 12(9), 1174. https://doi.org/10.3390/ani12091174.

Anonymous, 2005. Standard Methods for the Examination of Water and Waste Water. 23rd Edn. American Public Health Association, American Water Works Association, and Water Environment Federation. Available from: https://www.standardmethods.org/. Accessed on: 30 October 2025.

Anonymous, 2024. The State of World Fisheries and Aquaculture 2024: Blue transformation in action. Rome, FAO. Available from: https://openknowledge.fao.org/items/06690fd0-d133-424c-9673-1849e414543d.pdf. Accessed on: 30 October 2025.

Ardiansyah, A., Fotedar, R., 2016. Water quality, growth and stress responses of juvenile barramundi (*Lates calcarifer* Bloch), reared at four different densities in integrated recirculating aquaculture systems. Aquaculture 458,113–120. doi:10.1016/j.aquaculture.2016.03.001.

Ayyappan, S., Moza, U., Gopalakrishnan, D., Bharathiamma, M., Jena, J.K., Pandey, A.K., 2011. Handbook of fisheries and aquaculture. Indian Council of Agricultural Research, New Delhi. https://www.researchgate.net/publication/322821150_Handbook_of_Fisheries_and_Aquaculture.

Bais, B., 2018. Fish scenario in India with emphasis on Indian major carps. International Journal of Avian & Wildlife Biology 3(6), 409–411. https://doi.org/10.15406/ijawb.2018.03.00130.

Barcellos, L.G., Nicolaiewsky, S., De Souza, S.G., Lulhier, F., 2001. The effects of stocking density and social interaction on acute stress response in Nile tilapia *Oreochromis niloticus* (L.) fingerlings. Aquaculture Research 30(11–12), 887–892. http://dx.doi.org/10.1046/j.1365-2109.1999.00419.x.

Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases 1, 3–26. https://doi.org/10.1016/0959-8030(91)90019-G.

Bartonkova, J., Hyrsl, P., Vojtek, L., 2016. Glucose determination in fish plasma by two different moderate methods. Acta Veterinaria Brno 85 349–353. http://dx.doi.org/10.2754/avb201685040349.

Bi, B., Yin, Y., Zhao, Y., He, M., Song, H., Kong, L., Gao, Y., 2023. Effect of crowding stress on growth performance, the antioxidant system and humoral immunity in hybrid sturgeon. Aquaculture Reports 28, 101468. https://doi.org/10.1016/j.aqrep.2023.101468.

Brown, J.A., Watson, J., Bourhill, A., Wall, T., 2008. Evaluation and use of the Lactate Pro, a portable lactate meter, in monitoring the physiological well-being of farmed Atlantic cod (*Gadus morhua*). Aquaculture 285(1–4), 135–141. https://doi.org/10.1016/j.aquaculture.2008.08.027.

Carbonara, D.M., Corte, P.L., Parrinello, D., 2023. Fish welfare in aquaculture: Physiological and immunological activities for diets, social and spatial stress on Mediterranean aquacultured species. Fishes 8(8), 414.

Chatterjee, N., Pal, A.K., Das, T., Mohammed, M.S., Sarma, K., Venkateshwarlu, G., Mukherjee, S.C., 2006. Secondary stress responses in Indian major carps *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) and *Cirrhinus mrigala* (Hamilton) fry to increasing packing densities. Aquaculture Research 37(5), 472–476. https://doi.org/10.1111/j.1365-2109.2006.01469.x

Chen, G., Long, J., Li, H., Xu, J., Yuan, J., Yang, Q., Feng, L., Jiang, W., Wu, P., 2023. The Protective effect of a dietary extract of mulberry (*Morus alba* L.) leaves against a high stocking density, copper and trichlorfon in crucian carp (*Carassius auratus*). Animals 13(16), 2652. https://doi.org/10.3390/ani13162652.

Conte, F.S., 2004. Stress and the welfare of cultured fish. Applied Animal Behaviour Science 86(3–4), 205–223. https://doi.org/10.1016/j.applanim.2004.02.003.

Cox, D.K., 1974. Effects of the three heating rates on the critical thermal maximum of bluegill. In: Gibbons, J.W., Sharitz, R.R. (Eds.), Thermal ecology, AEC symposium series (Conf-73055), Springfield, pp. 150–163. https://www.scirp.org/reference/referencespapers?referenceid=854588.

Habib, S.A.A., Naji, A., Seyed Mortezaei, S.R., Sourinejad, I., Akbarzadeh, A., 2022. Effects of restricted feeding levels and stocking densities on water quality, growth performance, body composition and mucosal innate immunity of Nile tilapia (*Oreochromis niloticus*) fry in a biofloc system. Aquaculture 546, 737320. https://doi.org/10.1016/j.aquaculture.2021.737320.

Dai, Y., Shen, Y., Guo, J., Yang, H., Chen, F., Zhang, W., Wu, W., Xu, X., Li, J., 2024. Glycolysis and gluconeogenesis are involved in glucose metabolism adaptation during fasting and re-feeding in black carp (*Mylopharyngodon piceus*). Aquaculture and Fisheries 9(2), 226–233. https://doi.org/10.1016/j.aaf.2022.04.003.

Davison, W., Cooper, C.A., Sloman, K.A., Wilson, R.W., 2023. A method for measuring meaningful physiological variables in fish blood without surgical cannulation. Scientific Reports 13(1). https://doi.org/10.1038/s41598-023-28061-w.

Dawood, M.M.A.O., Kimera, F., 2022. A review on recirculating aquaculture system: Influence of stocking density on fish and crustacean behavior, growth performance, and immunity. Annals of Animal Science 22(3), 135–151. https://doi.org/10.2478/aoas-2022-0014.

Dediu, L., Docan, A., Cretu, M., Grecu, I., Mogodan, A., Maereanu, M., Oprea, L., 2021. Effects of stocking density on growth performance and stress responses of bester and bester ♀× beluga ♂ juveniles in recirculating aquaculture systems. Animals 11(8), 2292. https://doi.org/10.3390/ani11082292.

Dobsikova, R., Svobodova, Z., Blahova, J., Modra, H., Velisek, J., 2009. The effect of transport on biochemical and haematological indices of common carp (*Cyprinus carpio* L.). Czech Journal of Animal Science 54(11), 510–518. https://doi.org/10.17221/52/2009-CJAS.

Faudzi, N.M., Sobri, M.I., Othman, R., Ching, F.F., Shaleh, S.R.M., 2021. Water temperature and stocking density for long-hour transportation of hybrid grouper *Epinephelus fuscoguttatus* × *E. lanceolatus*. AACL Bioflux 14(2), 1098–1106. Available from: https://www.bioflux.com.ro/docs/2021.1098-1106.pdf.

Fiordelmondo, E., Magi, G.E., Mariotti, F., Bakiu, R., Roncarati, A., 2020. Improvement of the water quality in rainbow trout farming by means of the feeding type and management over 10 years (2009–2019). Animals 10(9), 1541. https://doi.org/10.3390/ani10091541.

Gomes, L.C., Roubach, R., Araujo-Lima, C.A., Chippari-Gomes, A.R., Lopes, N.P., Urbinati, E.C., 2003. Effect of fish density during transportation on stress and mortality of juvenile tambaqui *Colossoma macropomum*. Journal of the World Aquaculture Society 34(1), 76–84. http://dx.doi.org/10.1111/j.1749-7345.2003.tb00041.x.

Hassid, W.J., Abraham, S., 1957. Chemical procedures for analysis of polysaccharides. In: Calowick, S.P., Kaplan, N.O. (Eds.), Methods in enzymology. Academic Press, New York, USA, 3, 35–36. https://www.scirp.org/reference/referencespapers?referenceid=42231.

Hematyar, N., Rahimnejad, S., Waghmare, S.G., Malinovskyi, O., Policar, T., 2024. Effects of stocking density and pre-slaughter handling on the fillet quality of largemouth bass (*Micropterus salmoides*): Implications for fish welfare. Foods 13(10), 1477. https://doi.org/10.3390/foods13101477.

Hoseini, S.M., Ghelichpour, M., Hoseinifar, S.H., 2024. Sodium butyrate suppresses stress, immunosuppression and oxidative stress in common carp (*Cyprinus carpio*) reared at high stocking density. Aquaculture International 32, 10009–10028. https://doi.org/10.1007/s10499-024-01647-5.

Husen, Md.A., Sharma, S., 2014. Efficacy of anaesthetics for reducing stress in fish during aquaculture practices – A review. Kathmandu University Journal of Science, Engineering and Technology 10(1), 104–123. http://dx.doi.org/10.3126/kuset.v10i1.63691.

Husen, M.A., Mehta, S.N., Hussain, M.S., 2021. Fish seed transportation losses, its causes and good aquaculture practices (GAP) for fish seed transportation in Nepal. International Journal of Fisheries and Aquatic Research 6(2), 32. http://www.fishjournals.com/archives/2021/vol6/issue2/6-2-17.

Islam, M.N., Hossain, M.A., 2013. Mortality rate of fish seeds (*Labeo rohita*) during traditional transportation system in northwest Bangladesh. Journal of Scientific Research 5(2), 383–392. https://doi.org/10.3329/jsr.v5i2.10572.

Jia, R., Wang, L., Hou, Y., Feng, W., Li, B., Zhu, J., 2022. Effects of stocking density on the growth performance, physiological parameters, redox status and lipid metabolism of *Micropterus salmoides* in integrated rice–fish farming systems. Antioxidants 11(7), 1215. https://doi.org/10.3390/antiox11071215.

Jia, Y., Wang, J., Gao, Y., Huang, B., 2021. Hypoxia tolerance, haematological, and biochemical response in juvenile turbot (*Scophthalmus maximus* L). Aquaculture 535, 736380. https://doi.org/10.1016/j.aquaculture.2021.736380.

Kamalam, B.S., Patiyal, R.S., Rajesh, M., Mir, J.I., Singh, A.K., 2017. Prolonged transport of rainbow trout fingerlings in plastic bags: Optimization of hauling conditions based on survival and water chemistry. Aquaculture 480, 103–111. https://doi.org/10.1016/j.aquaculture.2017.08.012.

Klug, J., Treuting, P.M., Sanders, G.E., Winton, J.R., Kurath, G., 2021. Effects of stocking density on stress response and susceptibility to infectious hematopoietic necrosis virus in rainbow trout. Journal of the American Association for Laboratory Animal Science 60(6), 637–647. https://doi.org/10.30802/aalas-jaalas-21-000003.

Laiz-Carrión, R., Viana, I.R., Cejas, J.R., Ruiz-Jarabo, I., Jerez, S., Martos, J.A., Eduardo, A.B., Mancera, J.M., 2012. Influence of food deprivation and high stocking density on energetic metabolism and stress response in red porgy (*Pagrus pagrus* L). Aquaculture International 20, 585–599. http://dx.doi.org/10.1007/s10499-011-9488-y.

Li, Z., Gao, Q., Dong, S., Dong, K., Xu, Y., Mei, Y. and Hou, Z., 2024. Effects of chronic stress from high stocking density in mariculture: evaluations of growth performance and lipid metabolism of rainbow trout (*Oncorhynchus mykiss*). Biology (Basel) 13(4), 263. doi: 10.3390/biology13040263.

Lim, L.C., Dhert, P., Sorgeloos, P., 2003. Recent developments and improvements in ornamental fish packaging systems for air transport. Aquaculture Research 34, 923–935. https://doi.org/10.1046/j.1365-2109.2003.00946.x.

Liu, B., Xu, P., Brown, P.B., Xie, J., Ge, X., Miao, L., Zhou, Q., Ren, M., Pan, L., 2016. The effect of hyperthermia on liver histology, oxidative stress and disease resistance of the Wuchang bream (*Megalobrama amblycephala*). Fish and Shellfish Immunology 52, 317–324. DOI: 10.1016/j.fsi.2016.03.018.

Minahal, Q., Fatima, S., Komal, W., Liaqat, R., 2024. Effects of different stocking densities on growth, nutritional quality, stress and antioxidant response in Labeo rohita; cultured in in-pond raceway system. PLoS ONE 19(5), e0298753. https://doi.org/10.1371/journal.pone.0298753.

Mommsen, T.P., Vijayan, M.M., Moon, T.W., 1999. Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9(3), 211–268. https://doi.org/10.1023/A:1008924418720.

Nair, V.R., Parvathy, U., Jithin, T.J., Binsi, P.K., Ravishankar, C.N., 2023. Live transportation of food fishes: Current scenario and future prospects. Current Science 124(4), 418–425. https://doi.org/10.18520/cs/v124/i4/418-425.

Pakhira, C., Nagesh, T.S., Abraham, T.J., Dash, G., Behera, S., 2015. Stress responses in rohu, *Labeo rohita* transported at different densities. Aquaculture Reports 2, 39–45. https://doi.org/10.1016/j.aqrep.2015.06.002.

Paredes, O.R., Saravia, J., Morera, F.J., Muñoz, J.L.P., Ruiz-Jarabo, I., Mancera, J.M., Vargas-Chacoff, L., 2020. Stocking density affects the growth performance, intermediary metabolism, osmoregulation, and response to stress in Patagonian blennie (*Eleginops maclovinus*). Aquaculture 515, 734565. https://doi.org/10.1016/j.aquaculture.2019.734565.

Pickering, A.D., Pottinger, T.G., 1989. Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. Fish Physiology and Biochemistry 7(1–6), 253–258. https://doi.org/10.1007/BF00004714.

Polakof, S., Panserat, S., Soengas, J.L., Moon, T.W., 2012. Glucose metabolism in fish: A review. Journal of Comparative Physiology B 182, 1015–1045. https://doi.org/10.1007/s00360-012-0658-7.

Portz, D.E., Woodley, C.M., Cech, J.J., 2006. Stress-associated impacts of short-term holding on fishes. Reviews in Fish Biology and Fisheries 16, 125–170. http://dx.doi.org/10.1007/s11160-006-9012-z.

Putri, T.A., Maya, S., Santanumurti, M.B., 2020. Nile tilapia (*Oreochromis niloticus*) fish hatchery technique: The survival rate evaluation in IBAT Pandaan, Pasuruan, East Java. In: IOP Conference Series: Earth and Environmental Science 441(1), 012052. https://doi.org/10.1088/1755-1315/441/1/012052.

Ruane, N.M., Carballo, E.C., Komen, J., 2002. Increased stocking density influences the acute physiological stress response of common carp (*Cyprinus carpio* L). Aquaculture Research 33(10), 777–784. https://doi.org/10.1046/j.1365-2109.2002.00717.x.

Ruiz, N., García-Meilán, I., Khansari, A.R., Teles, M., Pastor, J., Tort, L., 2024. Repeated hypoxic episodes allow hematological and physiological habituation in rainbow trout. Frontiers in Physiology 15, 1289903. https://doi.org/10.3389/fphys.2024.1289903.

Schreck, C.B., Contreras-Sanchez, W., Fitzpatrick, M.S., 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197(1–4), 3–24. https://doi.org/10.1016/S0044-8486(01)00580-4.

Schreck, C.B., Jonsson, L., Feist, G., Reno, P.W., 1995. Conditioning improves performance of juvenile Chinook salmon, *Oncorhynchus tshawytscha*, to transportation stress. Aquaculture 135 (1–3), 99–106. https://doi.org/10.1016/0044-8486(95)01018-1.

Shah, M.I., 2021. Transportation of live fish seed. Fisheries and Aquaculture Journal. https://hal.archives-ouvertes.fr/hal-03627394.

Sousa, M.M.C., Coutinho, F., Castro, C., Fontinha, F., 2021. Functional feeds to tackle meagre (*Argyrosomus regius*) stress: Physiological responses under acute stressful handling conditions. Marine Drugs 19(11), 598. https://doi.org/10.3390/md19110598.

Suguna, 2020. Management of stress in culture fish. International Journal of Bio-resource and Stress Management 11(6), 607–612. HTTPS://DOI.ORG/10.23910/1.2020.2152a.

Sutphin, Z.A., Hueth, C.D., 2015. Effects of loading density during transport on physiological stress and survival of Sacramento-San Joaquin Delta fishes. California Fish and Game 101(2), 108–130.

Swain, H.S., Das, B.K., Upadhyay, A., Ramteke, M.H., Kumar, V., Meena, D.K., Sarkar, U.K., Chadha, N.K., Rawat, K., 2022. Stocking density mediated stress modulates growth attributes in cage reared *Labeo rohita* (Hamilton) using multifarious biomarker approach. Scientific Reports 12(1). https://doi.org/10.1038/s41598-022-13570-x.

Tan, C., Sun, D., Tan, H., Liu, W., Luo, G., Wei, X., 2018. Effects of stocking density on growth, body composition, digestive enzyme levels and blood biochemical parameters of *Anguilla marmorata* in a recirculating aquaculture system. Turkish Journal of Fisheries and Aquatic Sciences 18(1), 9–18. https://www.cabdirect.org/cabdirect/abstract/20173374745.

Thomas, P., Robertson, L., 1991. Plasma cortisol and glucose stress responses of red drum (*Sciaenops ocellatus*) to handling and shallow water stressors and anesthesia with MS-222, quinaldine sulfate and metomidate. Aquaculture 96(1), 69–76. https://doi.org/10.1016/0044-8486(91)90140-3.

Vaage, B.M., Liss, S.A., Fischer, E.S., Khan, F., Hughes, J.S., 2023. Can portable glucose and lactate meters be a useful tool in quantifying stress of juvenile Chinook salmon? Conservation Physiology 11(1). https://doi.org/10.1093/conphys/coad046.

Wells, M.G., Pankhurst, N.W., 1999. Evaluation of simple instruments for measuring blood glucose and lactate, and plasma protein as stress indicators in fish. Journal of the World Aquaculture Society 30(2), 276–284. https://doi.org/10.1111/j.1749-7345.1999.tb00876.x.

Zhang, H., Wang, Q., Dong, Y., Mei, J., Xie, J., 2023. Effects of tricaine methanesulphonate (MS-222) on physiological stress and fresh quality of sea bass (*Lateolabrax maculatus*) under simulated high-density and long-distance transport stress. Biology 12(2), 223. https://doi.org/10.3390/biology12020223.