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# Role of Pre-transport Conditioning Density in Enhancing Transportation Success of *Labeo rohita* Fingerlings

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## ABSTRACT

The present study was conducted during September, 2025 at the Fish Seed Production Centre and Instructional Fish Farm, College of Fisheries Science, Nagpur, Maharashtra, India, to evaluate the effects of different pre-transport conditioning densities on the physiological responses and transportation performance of *Labeo rohita* fingerlings. Fingerlings were conditioned for 24 h in a flow-through system at four stocking densities. Blood glucose, lactate, muscle glycogen, haemoglobin, haematocrit, water quality, gut evacuation time, and survival were assessed during conditioning and after manual simulated transportation for 12 and 24 h with 4 fingerlings $l^{-1}$. Glucose and lactate levels declined progressively during conditioning, while glycogen increased, indicating metabolic recovery. Haemoglobin and haematocrit decreased during conditioning but increased after transportation, reflecting adaptive responses to transport stress. Water quality remained suitable during conditioning, although ammonia increased and dissolved oxygen declined following transportation. Gut evacuation time decreased significantly with increasing stocking density, from 23.0±0.71 h in $T_1$ to 18.4±1.14 h in $T_4$. Survival declined with increasing conditioning density and transport duration, with the lowest values observed in $T_4$ (4 fingerlings $l^{-1}$). Although $T_1$ (1 fingerling $l^{-1}$) recorded the highest survival, $T_3$ provided comparable physiological stability, satisfactory post-conditioning and post-transport survival, and efficient conditioning density. Therefore, conditioning *Labeo rohita* at 3 fingerlings $l^{-1}$ for 19.80 h is recommended as the optimum strategy for improving transportation success and operational efficiency in commercial hatcheries.

**KEYWORDS:** *Labeo rohita*, conditioning density, transportation stress, gut evacuation, survival

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***Data Availability Statement:*** Legal restrictions are imposed on the public sharing of raw data. However, author 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

Aquaculture is one of the fastest-growing food production sectors globally, with an annual growth rate of 8.3% between 1970 and 2008, exceeding that of capture fisheries and livestock production (Anonymous, 2024). India ranks as the second-largest fish-producing nation, contributing approximately 8% of global fish production (Anonymous, 2025). Inland aquaculture drives this expansion, with freshwater species accounting for more than 95% of total aquaculture output (Reddy, 2022; Anonymous, 2025). Among freshwater species, Indian Major Carps (IMCs) constitute the primary production base (Jayasankar, 2018). *Labeo rohita* (rohu) is the leading contributor to inland fish production in India, accounting for approximately 38.20% of total inland output (Anonymous, 2025). Owing to its rapid growth, compatibility in polyculture systems, and high consumer preference, rohu represents a cornerstone species in freshwater aquaculture. The continued expansion of rohu farming depends substantially on the availability of healthy, stress-resilient fingerlings and their efficient transportation from hatcheries to grow-out facilities. Transportation of live fish seed is an inevitable and highly stress-prone process involving handling, crowding, confinement, and water quality deterioration. Mortality during carp seed transport may reach 5–10% (Bisht et al., 2013), while sub-lethal stress impairs physiological stability and subsequent performance. Packing density is a key determinant of transport success. Although higher stocking densities improve transport efficiency and reduce operational costs, excessive crowding causes hypoxia, ammonia accumulation, water quality deterioration, and physiological stress responses (Carneiro and Urbinati, 2002; Urbinati et al., 2003; Singh et al., 2004). Optimal stocking densities minimise stress, improve post-transport survival, reduce disease risk, whereas high stocking density coupled with poor water quality compromises fish health and increases transport-related losses (Suguna, 2020). Beyond transport density, the physiological status of fingerlings before loading plays a crucial role in transport tolerance. Pre-transport conditioning, particularly conditioning density, significantly influences stress responsiveness and metabolic preparedness. Higher conditioning densities increase dissolved oxygen depletion and total ammonia nitrogen accumulation, reflecting greater metabolic waste production and oxygen demand (Shelke et al., 2026). Conditioning density also modulates blood glucose, lactate, haemoglobin, and haematocrit, indicating the adaptive capacity of fish under crowding stress. Blood glucose and lactate are established stress biomarkers that increase through catecholamine- and cortisol-mediated energy mobilisation and enhanced anaerobic glycolysis during acute stress (Perry and Reid, 1993; Husen and Sharma, 2015; Fazio et al., 2015). Likewise, haemoglobin and haematocrit vary with oxygen transport demands, reflecting compensatory metabolic adjustments (Davison et al., 2023). Gut evacuation constitutes another essential aspect of conditioning. Inadequate gut clearance elevates metabolic waste release and ammonia accumulation during confinement, thereby compromising water quality and survival during transport (Conte, 2004; Nair et al., 2023). Monitoring gut evacuation time thus provides an important indicator of transport readiness and physiological preparedness. Despite the recognised importance of conditioning, most available studies focus primarily on immediate transport survival and rarely integrate pre-transport stocking density, physiological stress responses, and transportation performance in a comprehensive framework. Species-specific data for *Labeo rohita* fingerlings under varying conditioning densities remain limited. Therefore, the present study investigated the influence of pre-transport conditioning density on metabolic (blood glucose, blood lactate, muscle glycogen), haematological (haemoglobin, haematocrit), and gut evacuation responses of *Labeo rohita* fingerlings, and evaluated how these physiological alterations affected subsequent transportation performance.

## 2. MATERIALS AND METHODS

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

The experiment was conducted at the Fish Seed Production Centre and instructional Fish Farm of the College of Fisheries Science, Nagpur, Maharashtra, India (21°09'11.6'N, 79°02'57.1'E). Advanced fry of *Labeo rohita* measuring 40–60 mm in total length were obtained from a commercial hatchery and seed rearing unit. After procurement, the fry were stocked in an earthen nursery pond at a density of 20–30 fry $m^{-2}$ in accordance with standard management practices (Ayyappan et al., 2011). The fish were reared under semi-intensive conditions and fed a formulated diet containing 35% crude protein. Within approximately 30 days, the fry reached 80–100 mm in length and were classified as fingerlings. These fingerlings were harvested and utilized for the pre-transport conditioning experiment.

### *2.2. Experimental design and conditioning procedure*

The conditioning experiment was performed using twenty circular FRP tanks, each with a total capacity of 1000 l, operated under a continuous flow-through system. The effective water volume in each tank was maintained at 500 l, with a regulated water exchange rate of approximately 6.5 l $min^{-1}$. Rohu fingerlings were conditioned at four stocking densities of 1, 2, 3, and 4 fingerlings $l^{-1}$, designated as $T_1$, $T_2$, $T_3$, and $T_4$, respectively. Each treatment was conducted in five replicates. The conditioning period extended for 24 h, during which fish were maintained without feeding and

continuously observed. During conditioning, when each treatment showed gut-emptying point from that point, simulated transportation was started with a packing density of 4 fingerlings $l^{-1}$ for 12 h and 24 h.

*2.3. Sampling schedule and physiological assessments*

Physiological and haematological parameters were evaluated at 1, 4, 8, 12, 16, 20 and 24 h during the conditioning period and after simulated transportation. The variables analysed included blood glucose, blood lactate, muscle glycogen, haemoglobin (HB), and haematocrit (HCT). These indicators were selected to assess metabolic responses, stress adaptation, and oxygen transport capacity under varying conditioning densities.

*2.4. Evaluation of gut evacuation, fish behaviour, and mortality*

Fish behaviour and mortality were recorded at 4 h intervals throughout the conditioning period. Fingerlings exhibiting reduced swimming activity, diminished response to gentle tactile stimulation, or abnormal swimming behaviour were categorised as weak. Gut evacuation time was assessed visually based on the disappearance of faecal matter in tank water, and the duration required for complete clearance was documented to determine transport readiness. Mortalities were recorded at each observation interval, and dead fish were immediately removed to prevent deterioration of water quality.

*2.5. Water quality monitoring*

Before initiating the experiment, baseline water quality parameters were measured to ensure uniform conditions across all tanks. The parameters included temperature, pH, dissolved oxygen (DO), total alkalinity, total hardness, total ammonia nitrogen (TAN), and free carbon dioxide ($CO_2$). During the 24 h conditioning period, these parameters were monitored at 4 h intervals to ensure that water quality remained within acceptable ranges for rohu fingerlings. Water temperature was measured using a multi-stem thermometer, pH was recorded with a Thermo Scientific digital pH meter, and dissolved oxygen was determined using an Aquasol AM-DO-01 meter. Total alkalinity was estimated using a Hanna Instrument HI775 kit. Total ammonia nitrogen was analysed spectrophotometrically, whereas free $CO_2$ and total hardness were determined titrimetrically following standard procedures outlined by Anonymous (2005).

*2.6. Collection and analysis of blood and tissue samples*

Before sampling, fingerlings were anaesthetised using clove oil at a concentration of 50 µl $l^{-1}$ to minimise handling stress. Blood samples were collected via caudal venipuncture using 1 mL heparinised syringes and transferred into EDTA-coated microcentrifuge tubes. Approximately 100–120 µl of blood was collected from each fingerling for analysis. Blood glucose levels were determined using a portable glucometer (OneTouch Ultra) following Bartonkova et al. (2017). Haemoglobin concentration was estimated using the cyanmethemoglobin method with Drabkin's reagent (Qualigen). In this procedure, 20 µl of blood was mixed with Drabkin's working solution, and absorbance was recorded at 540 nm using a UV–VIS spectrophotometer. Haemoglobin values were calculated by comparison with a standard cyanmethemoglobin solution. Haematocrit was measured by centrifuging heparinised capillary tubes containing approximately 65–70 µl of blood to determine packed cell volume. Blood lactate levels were quantified using a portable lactate meter (Lacto-spark) in accordance with established protocols (Wells and Pankhurst, 1999; Brown et al., 2008; Vaage et al., 2023). For muscle glycogen estimation, anaesthetised fish were euthanised by decapitation, and muscle tissues were collected. The samples were digested in 30% potassium hydroxide (KOH), precipitated with 95% ethanol, and glycogen concentration was determined using the anthrone method described by Hassid and Abraham (1957).

*2.7. Statistical analysis*

Percentage data relating to survival were transformed with logit transformation method prior to statistical analysis to stabilize variance and ensure normal distribution. The transformed data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test to identify significant differences among treatments at $p<0.05$. Statistical analyses were performed using SPSS software (Version 16.0).

## 3. RESULTS AND DISCUSSION

*3.1. Influence of conditioning on metabolic parameters*

*3.1.1. Blood glucose*

Transportation and handling are among the most stressful events experienced by fish. Plasma glucose is a well-established primary stress indicator, reflecting activation of the hypothalamic–pituitary–interrenal (HPI) axis and subsequent cortisol-mediated gluconeogenesis (Barton, 2002; Iwama et al., 2005).

In the present study, *Labeo rohita* fingerlings exhibited elevated glucose levels at 1 h of conditioning (~103–105 mg $dl^{-1}$), exceeding the normal physiological range of 40–90 mg $dl^{-1}$. This hyperglycaemic response confirms acute stress associated with netting, handling, hauling, and crowding during transfer to the conditioning tanks. Such elevation reflects rapid mobilisation of energy reserves and increased metabolic activity following acute stress (Wendelaar Bonga, 1997; Barton, 2002), mediated through activation of the HPI axis and stimulation of glycogenolysis and gluconeogenesis (Barton and Iwama, 1991).

As presented in Figure 1, glucose concentrations declined progressively from 1 h to 24 h across all stocking densities ($T_1$–$T_4$). By 24 h, values decreased to approximately 30–36 mg $dl^{-1}$, representing nearly a 70% reduction from initial levels. The decline was highly linear, as illustrated in Figure 2 ($R^2$ ≈0.986–0.989), demonstrating a strong negative relationship between conditioning duration and blood glucose levels. This consistent reduction indicates recovery from acute handling stress and restoration of metabolic balance under fasting and stable flow-through conditions. Although all treatments followed a similar downward trajectory, fish maintained at the highest density ($T_4$) retained comparatively elevated glucose levels during intermediate stages, suggesting relatively prolonged stress under increased crowding.

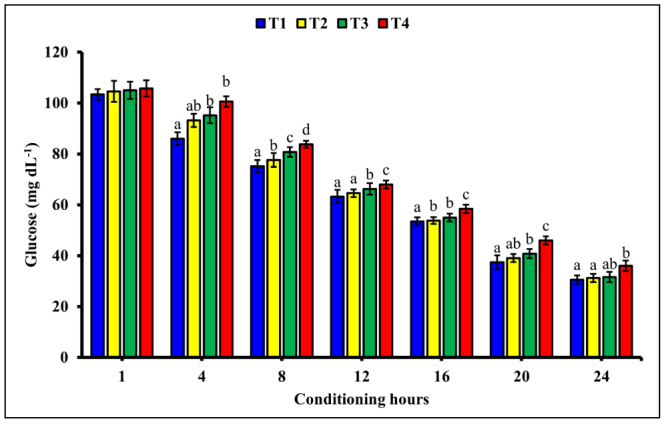


Figure 1: Glucose level in rohu fingerlings conditioned for up to 24 h at different stocking densities prior to transportation. Fingerlings were stocked at $T_1$ (1 fingerling $l^{-1}$), $T_2$ (2 fingerlings $l^{-1}$), $T_3$ (3 fingerlings $l^{-1}$), and $T_4$ (4 fingerlings $l^{-1}$), and sampled at 1, 4, 8, 12, 16, 20, and 24 h. Values are mean±SE; different superscripts within a time point indicate significant differences among treatments ($p \leq 0.05$)

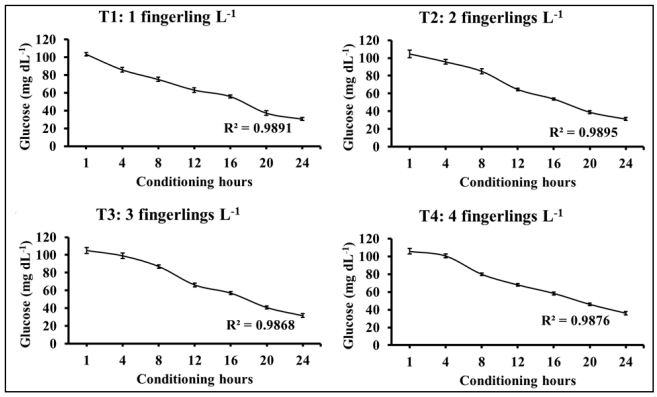


Figure 2: Changes in glucose levels of rohu fingerlings during 24 h conditioning at varying stocking densities prior to transportation. Fingerlings were stocked at $T_1$ (1 fingerling $l^{-1}$), $T_2$ (2 fingerlings $l^{-1}$), $T_3$ (3 fingerlings $l^{-1}$), and $T_4$ (4 fingerlings $l^{-1}$), and sampled at 1, 4, 8, 12, 16, 20, and 24 h. Values are mean±SE

Following 12 h of simulated transportation, glucose concentrations increased significantly across treatments, ranging from 128.80±3.27 mg $dl^{-1}$ in $T_1$ to 138.20±3.63 mg $dl^{-1}$ in $T_4$. The highest conditioning density recorded significantly greater glucose concentrations, whereas T3 showed intermediate values and did not differ significantly from $T_1$ and $T_2$. After 24 h of simulated transportation, glucose levels increased further, ranging from 142.00±2.92 mg $dl^{-1}$ in $T_1$ to 149.20±2.39 mg $dl^{-1}$ in $T_4$, indicating cumulative stress under prolonged confinement. Again, $T_4$ exhibited significantly higher glucose concentrations, while $T_3$ maintained moderate values statistically comparable to lower-density treatments.

Overall, plasma glucose exhibited a clear three-phase response: initial hyperglycemia due to handling stress, progressive decline during conditioning indicating recovery, and renewed elevation during simulated transportation. The moderate glucose response observed in T3 during both conditioning and transportation, combined with its higher stocking capacity relative to $T_1$ and $T_2$, indicates that 3 fingerlings $l^{-1}$ represents an optimal pre-transport conditioning density for rohu fingerlings.

### *3.1.2. Lactate level*

Blood lactate is a sensitive indicator of anaerobic metabolism and acute stress in fish. Under unstressed conditions, freshwater species generally exhibit lactate levels of 1–3 mmol $l^{-1}$ (Wells and Pankhurst, 1999). In the present study, *Labeo rohita* fingerlings showed elevated lactate concentrations at 1 h of conditioning (5.2–6.2 mmol $l^{-1}$), indicating acute stress due to netting, hauling, and crowding. This rise reflects enhanced anaerobic glycolysis triggered by activation of the HPI axis and catecholamine release (Barton and Iwama, 1991; Barton, 2002). Similarly, Santos et al. (2010) studied European seabass (*Dicentrarchus labrax*) and reported a significant increase in plasma lactate following acute net confinement stress, confirming lactate as a rapid indicator of handling stress.

Lactate levels declined progressively from 1 h to 24 h across all stocking densities (Figure 3), reaching 2.7–3.0 mmol $l^{-1}$ by 24 h, close to baseline values. The early phase (1–8 h) showed relatively higher lactate in $T_3$ and $T_4$, but differences diminished later. Regression analysis (Figure 3.4) demonstrated a strong negative relationship between conditioning duration and lactate, indicating metabolic recovery.

Comparable trends have been reported in other teleosts. Veiseth et al. (2006) observed rapid lactate elevation in Atlantic salmon after crowding, followed by normalisation, while Bi et al. (2023) reported transient metabolic increases in hybrid sturgeon under crowding stress, followed by stabilisation.

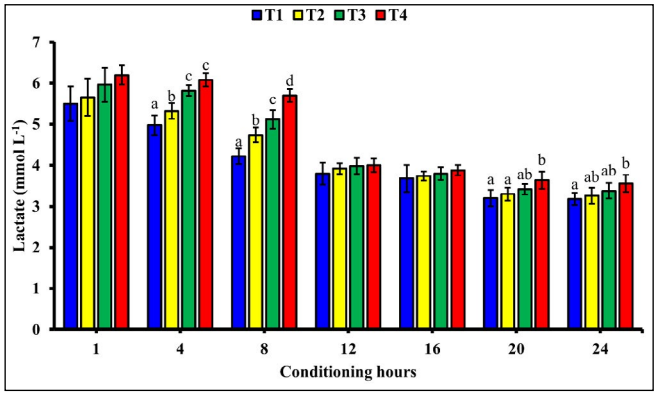


Figure 3: Lactate levels in rohu fingerlings conditioned for up to 24 h at different stocking densities prior to transportation. Fingerlings were stocked at $T_1$ (1 fingerling $l^{-1}$), $T_2$ (2 fingerlings $l^{-1}$), $T_3$ (3 fingerlings $l^{-1}$), and $T_4$ (4 fingerlings $l^{-1}$), and sampled at 1, 4, 8, 12, 16, 20, and 24 h. Values are mean±SE; different superscripts within a time point indicate significant differences among treatments ($p \leq 0.05$)

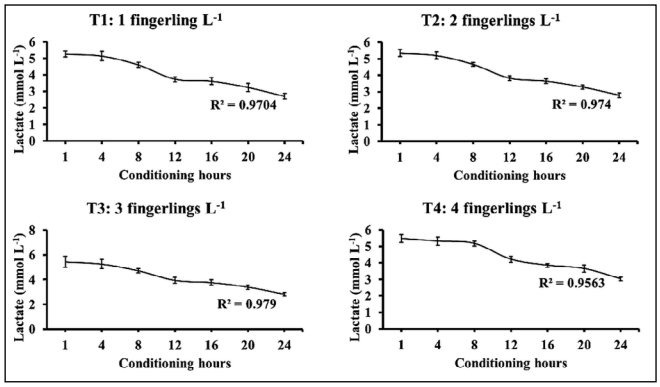


Figure 4: Changes in lactate levels of rohu fingerlings during 24 h conditioning at varying stocking densities prior to transportation. Fingerlings were stocked at $T_1$ (1 fingerling $l^{-1}$), $T_2$ (2 fingerlings $l^{-1}$), $T_3$ (3 fingerlings $l^{-1}$), and $T_4$ (4 fingerlings $l^{-1}$), and sampled at 1, 4, 8, 12, 16, 20, and 24 h. Values are mean±SE

Overall, the steady reduction in lactate suggests effective recovery during conditioning. The balanced decline observed in $T_3$ indicates that 3 fingerlings $l^{-1}$ provides an optimal density, supporting metabolic stabilisation without excessive crowding stress.

*3.1.3. Changes in glycogen level*

Muscle glycogen content in *Labeo rohita* exhibited an inverse relationship with plasma glucose during the 24 h conditioning period (Figure 5). At 1 h, glycogen levels were relatively low (≈218–222 µg $mg^{-1}$), reflecting rapid energy mobilisation following handling and crowding stress. As conditioning progressed, glycogen increased steadily,

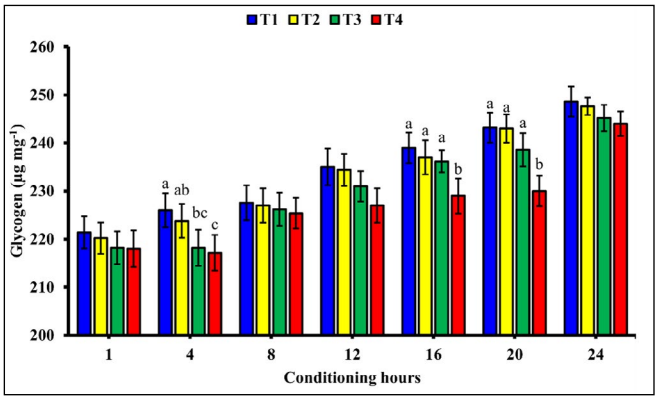


Figure 5: Glycogen concentrations in rohu fingerlings conditioned for up to 24 h at different stocking densities before transportation. Fingerlings were stocked at $T_1$ (1 fingerling $l^{-1}$), $T_2$ (2 fingerlings $l^{-1}$), $T_3$ (3 fingerlings $l^{-1}$), and $T_4$ (4 fingerlings $l^{-1}$), and glycogen levels were evaluated at 1, 4, 8, 12, 16, 20, and 24 h. Values are mean±SE; different superscripts within a time point indicate significant differences among treatments ($p \leq 0.05$)

reaching ≈244–249 µg $mg^{-1}$ by 24 h. Regression analysis (Figure 6) confirmed a strong positive relationship between conditioning time and glycogen levels ($R^2$=0.9491–0.9834), indicating restoration of energy reserves.

The increase was more evident in $T_1$–$T_3$, suggesting effective replenishment once acute stress subsided (Urbinati et al., 2003), whereas $T_4$ showed comparatively slower recovery. $T_3$ displayed a stable and balanced glycogen restoration pattern.

After simulated transportation (12 h and 24 h), glycogen reserves were utilised to meet elevated metabolic demands, resulting in reduced levels compared to pre-transport values,

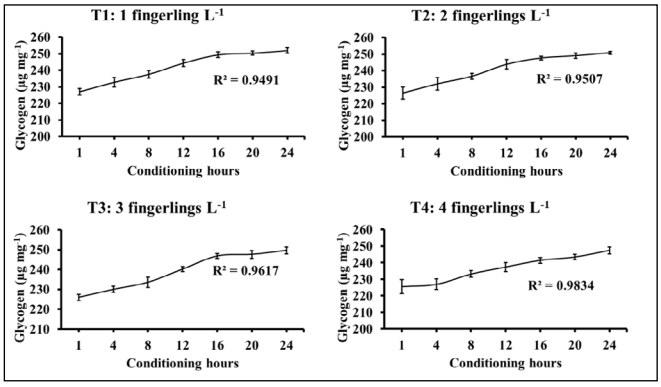


Figure 6: Changes in glycogen concentration of rohu fingerlings during 24 h conditioning at varying stocking densities before transportation. Fingerlings were stocked at $T_1$ (1 fingerling $l^{-1}$), $T_2$ (2 fingerlings $l^{-1}$), $T_3$ (3 fingerlings $l^{-1}$), and $T_4$ (4 fingerlings $l^{-1}$), and glycogen levels were evaluated at 1, 4, 8, 12, 16, 20, and 24 h. Data are expressed as mean±S.E

with greater depletion after 24 h. This reflects mobilization of stored carbohydrates during confinement stress.

Similar responses have been reported in other species. Trenzado and Carrick (2003) observed glycogen depletion followed by recovery in common carp (*Cyprinus carpio*). Barton et al. (2004) reported rapid glycogen utilization in dentex under handling stress, and Chang et al. (2007) documented glycogen depletion followed by stabilization in *Oreochromis mossambicus* during acclimation.

Overall, glycogen dynamics indicate energy restoration during conditioning and mobilization during transport, with $T_3$ showing the most balanced metabolic response.

### *3.2. Changes in haematological parameters*

#### *3.2.1. HB and HCT levels*

Conditioning influenced haematological responses in *Labeo rohita* fingerlings by modulating oxygen transport capacity under handling and crowding stress. Haemoglobin (HB) levels remained relatively stable during the first 8–12 h (≈9.8–10.3 g $dl^{-1}$) and gradually declined toward 24 h, reaching ~8.06–8.36 g $dl^{-1}$. These values are within the normal physiological range of 5–16 g $dl^{-1}$ (Chen and Luo, 2023). The decline likely reflects reduced oxygen demand under fasting and confinement, along with energy-conserving adjustments (Wedemeyer, 1990; Nikinmaa and Salama, 1998). Regression analysis confirmed a strong time-dependent decrease.

Haematocrit (HCT) showed a comparable trend, remaining stable at 28–31% up to 8 h and declining to ~21–24% by 24 h. This reduction may indicate modulation of erythrocyte volume or count under stress (Martinez-Porchas and Martinez-Cordova, 2012; He et al., 2015; Dagoudo et al., 2021), yet values remained within the reference range of 14–44%. Regression plots indicated strong negative relationships with conditioning time ($R^2$=0.9358–0.9728).

After simulated transportation, both HB and HCT increased compared to post-conditioning levels, reflecting compensatory responses to oxygen debt. The rise was observed after 12 h transport and was more pronounced after 24 h, indicating greater metabolic demand under prolonged confinement.

Overall, HB and HCT declined during conditioning but increased following transport stress, demonstrating adaptive haematological regulation in *Labeo rohita*.

### *3.3. Variation in water quality parameters*

Water quality is critical for successful conditioning and transportation, as poor conditions can intensify physiological stress (Boyd, 2017). Conditioning under a flow-through system helps maintain stable water quality and adequate dissolved oxygen (DO), and is recommended as a best management practice (Anonymous, 2001; Anonymous, 2006).

During conditioning, temperature, pH, alkalinity, and hardness remained stable across stocking densities, indicating effective system management. However, total ammonia nitrogen (TAN) showed density- and time-dependent variations. TAN was undetectable at 1 h but became measurable by 4 h, with $T_4$ recording the highest value (0.07±0.01 mg $l^{-1}$). Thereafter, TAN increased progressively with stocking density and time (Figure 7B). Since ammonia is the main nitrogenous waste in fish and can impair gill function and metabolism (Yuen and Chew, 2010), its accumulation is a concern. Similar density-related increases in ammonia have been reported previously (Saether et al., 2016; Honnananda et al., 2020). Nevertheless, TAN levels during conditioning remained below commonly cited toxic thresholds (0.2–2.0 mg $l^{-1}$), confirming acceptable water quality.

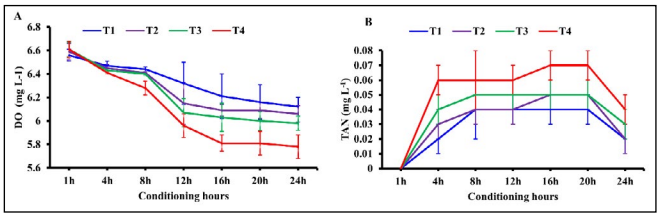


Figure 7: Changes in water quality parameters during the 24 h conditioning period of Labeo rohita fingerlings at different stocking densities ($T_1$: 1 fingerling $l^{-1}$, $T_2$: 2 fingerlings $l^{-1}$, $T_3$: 3 fingerlings $l^{-1}$, $T_4$: 4 fingerlings $l^{-1}$). (A) Dissolved oxygen (DO, mg $l^{-1}$). (B) Total ammonia nitrogen (TAN, mg $l^{-1}$). Data are expressed as mean±S.D

After simulated transportation, water quality deterioration was more evident. DO levels declined to below 4 mg $l^{-1}$ in both 12 h and 24 h transport groups, indicating increased oxygen demand under confinement. TAN concentrations peaked immediately after transport, ranging from ~1.28–1.40 mg $l^{-1}$ after 12 h and increasing to ~2.6–2.76 mg $l^{-1}$ after 24 h. The higher TAN in the 24 h group reflects greater ammonia accumulation during prolonged transport.

Overall, conditioning maintained stable water quality, while simulated transportation caused reduced DO and elevated TAN, particularly after 24 h. These results highlight the effects of transport duration and stocking density on water-quality stress in *Labeo rohita* fingerlings.

### *3.4. Influence of conditioning of fish on gut evacuation time and survival*

Gut evacuation time in *Labeo rohita* exhibited a clear density-dependent decline (Figure 8A–B). As stocking density increased from $T_1$ to $T_4$, gut evacuation time decreased from 23.00±0.71 h ($T_1$) to 18.4±1.14 h ($T_4$).

The regression analysis (Figure 8A) showed a negative relationship between stocking density and evacuation time, confirming that increased crowding accelerated gut clearance. The bar representation (Figure 8B) further indicates that $T_1$ differed significantly from higher densities, while $T_2$ and $T_3$ remained statistically comparable, and $T_4$ showed the shortest evacuation time. Elevated stocking density is known to disrupt gastrointestinal regulation (Liu et al., 2018; Alvarez et al., 2022). Cortisol-mediated stress alters intestinal integrity (Sadoul and Vijayan, 2016) and enhances gut motility (Jhingran and Pullin, 1985; Barton et al., 1987), leading to faster evacuation and potentially reduced nutrient assimilation (Jobling, 1981; Miegel et al., 2010; Ni et al., 2021). In contrast, longer evacuation times at lower densities favor digestive stability (Pandlan, 1967).

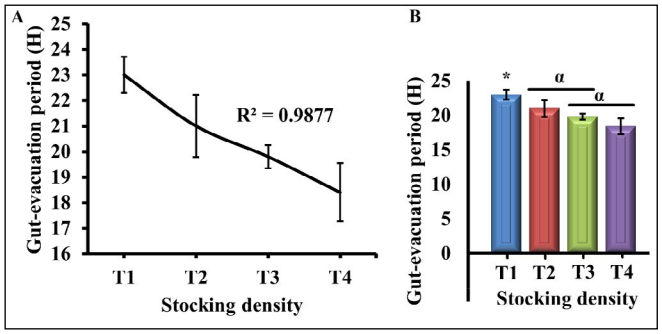


Figure 8: Effect of conditioning stocking density on gut evacuation time (H) of rohu fingerlings. (A) Regression analysis showing the negative relationship between stocking density and gut evacuation period. (B) Mean gut evacuation time across stocking densities ($T_1$–$T_4$), with bars representing mean±SE; Values are expressed as mean±S.D. $p<0.05$ is considered a significant difference. *Indicate significant difference from $T_1$, $T_2$ and $T_3$ group. α indicates non-significance among the groups ($p>0.05$)

Survival during conditioning remained high (>90%) across densities (Figure 9A). At the gut-emptying stage, $T_1$–$T_3$ maintained comparable survival (~92–93%), whereas $T_4$ showed a significant reduction (~90%). A similar pattern was observed after 24 h conditioning, with T4 again recording lower survival. Trend analysis (Figure 9B) revealed a progressive decline in survival with increasing stocking density under both gut-empty and 24 h conditions ($R^2$=0.8134 and 0.7472, respectively), confirming a density-dependent effect. These findings align with previous reports indicating that excessive crowding elevates stress and may compromise survival (Barton, 2002; Ramesh and Saravanan, 2008; Anders et al., 2021; Ebrahimzadeh Mousavi et al., 2023).

Following simulated transportation, survival declined in a duration-dependent manner. After 12 h transport, survival ranged from 96.67±1.86% ($T_1$) to 89.17±2.28% ($T_4$), with $T_4$ significantly lower than other treatments. After 24 h of transport, survival decreased further to 84.17±3.49% ($T_1$) and 75.83±3.49% ($T_4$), indicating cumulative stress under prolonged confinement. Although $T_1$ recorded the highest survival, $T_3$ maintained values statistically comparable to intermediate treatments, demonstrating balanced tolerance at moderate density.

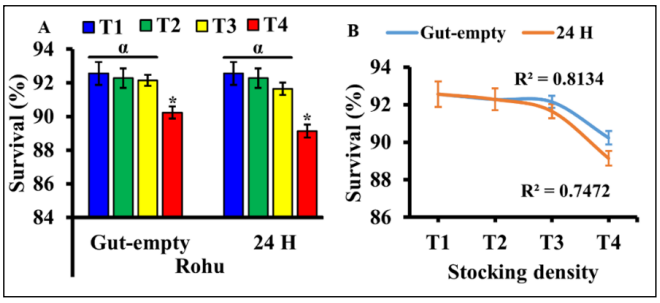


Figure 9: Influence of conditioning stocking density on survival (%) of rohu fingerlings. (A) Survival (%) at gut-emptying stage and after 24 h of conditioning across four stocking densities ($T_1$: 1 fingerling $l^{-1}$, $T_2$: 2 fingerlings $l^{-1}$, $T_3$: 3 fingerlings $l^{-1}$, $T_4$: 4 fingerlings $l^{-1}$). Values are expressed as mean±S.D. $p<0.05$ is considered as significant difference. * Indicate significant difference from $T_1$, $T_2$ and $T_3$ group. α Indicate non-significance among the groups ($p>0.05$). (B) Trend analysis showing density-dependent decline in survival under gut-empty and 24 h conditioning states

Collectively, Figure 8 and Figure 9 illustrate that increasing stocking density accelerates gut evacuation and gradually reduces survival, particularly at $T_4$. Conditioning at 3 fingerlings $l^{-1}$ (T3) provided an optimal compromise between efficient space utilization, acceptable gut evacuation time (~19.8 h), and relatively high survival, supporting its suitability for pre-transport conditioning in *Labeo rohita* fingerlings.

## 4. CONCLUSION

Pre-transport conditioning density influenced physiological status, gut evacuation, water quality, and transport performance of Labeo rohita fingerlings. Conditioning reduced handling stress, as shown by declining glucose and lactate and recovery of muscle glycogen. Haemoglobin and haematocrit declined during conditioning but increased after transportation. Higher densities accelerated gut evacuation but reduced survival, particularly during prolonged transport, with reduced dissolved oxygen and increased total ammonia nitrogen. Although $T_1$ showed the highest survival, $T_3$ (3 fingerlings $l^{-1}$) provided comparable stability, efficient space use, acceptable survival, and 19.8 h gut evacuation. Thus, 3 fingerlings $l^{-1}$ is recommended.

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