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Physiological Stress Responses and Recovery Dynamics of *Labeo rohita* (Hamilton, 1822) Fingerlings under Varying Packing Densities and Transport Durations

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ABSTRACT

The study was conducted during September–October, 2024 at the Fish Seed Production Center and Fish Farm, College of Fisheries Science, Nagpur, Maharashtra, to evaluate the effects of varied densities during transportation of *Labeo rohita* fingerlings on stress and mortality. Two transport durations (12 h and 24 h) were tested under five and seven packing densities, respectively. Post-transport, fingerlings were reared in hapas at a uniform density for 24 h, 48 h, 1 week, and 2 weeks to monitor stress recovery through physiological and hematological assessments. Transportation significantly elevated glucose and lactate levels, with the highest glucose recorded at higher densities: 147.50 mg dl^{-1} in 8 fingerlings l^{-1} (12 h) and 144.00 mg dl^{-1} in 4 fingerlings l^{-1} (24 h). Corresponding lactate concentrations were lower in 6 fingerlings l^{-1} (6.38±0.28 mmol l^{-1}) and 3 fingerlings l^{-1} (6.07±0.06 mmol l^{-1}), suggesting reduced anaerobic stress. Dissolved oxygen levels approached hypoxic limits: 3.36±0.25 mg l^{-1} (6 fingerlings l^{-1}) and 3.01±0.06 mg l^{-1} (3 fingerlings l^{-1}), while total ammonia nitrogen remained lower in these groups. Mortality exhibited a density-dependent pattern, with 6 fingerlings l^{-1} (12 h) showing only 5% mortality at 48 h post-transport, compared to ~15% in 8 fingerlings l^{-1}, primarily due to physical stress and post-transport weakness. Overall, 6 fingerlings l^{-1} for 12 h and 3 fingerlings l^{-1} for 24 h were identified as optimal densities. Extended post-transport monitoring revealed delayed stress and mortality responses that were often overlooked in short-term studies.

KEYWORDS: Fingerlings, packing density, rohu, stress, transportation

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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

India's diverse agroclimatic conditions and vast network of rivers, lakes, ponds, and reservoirs make it a key player in the global aquaculture sector, especially in freshwater fish production. Aquaculture is rapidly growing at over 10% annually, producing ~17 M tons and contributing ~1.1% to the national GDP (Anonymous, 2022a; Anonymous, 2022b). India now ranks second globally in aquaculture production, driven by smallholder farmers and commercial enterprises adopting advanced technologies. Aquaculture contributes 68% of India's fish production, forming a vital part of the fisheries sector (Anonymous, 2025; Reddy, 2022). Major farmed species include Indian major carps (IMC), minor carps, tilapia, and pangasius in freshwater, and penaeid shrimp in brackish water, while most freshwater fish is consumed domestically (Jayasankar, 2018; Anonymous, 2021). India has emerged as a leading producer of farmed shrimp, contributing 20% share in global shrimp exports (Rajani and Balasubramanian, 2025). These developments stem from advances in hatchery technologies, selective breeding, improved feeds, and innovative farm management practices. Transportation of fish fingerlings remains a crucial link supporting aquaculture expansion across India. Currently, both open (plastic bags with oxygen) and closed (aerated or recirculating tanks) systems are used, but survival rates vary with species, transport duration, stocking density, and handling. IMCs, Rohu (*Labeo rohita*), Catla (*Labeo catla*), and Mrigal (*Cirrhinus mrigala*), constitute ~85% of India's freshwater fish production (Anonymous, 2025). Among them, Rohu is particularly important due to its fast growth, adaptability, and high market demand. It is widely cultured in mono- and polyculture systems and contributes significantly to aquaculture income. Rohu farming success depends on healthy fingerling availability, as seed quality affects survival, growth, and disease resistance. Hatchery practices have advanced through induced breeding and regulated seed production. However, the transportation of delicate fingerlings remains a bottleneck. Transport stress affects survival and later growth through handling, temperature changes, crowding, oxygen depletion, and water quality shifts (Biswalet al., 2021b; Hasan and Bart, 2007; Pakhira et al., 2015). Among these, stocking or packing density is most critical. Optimizing density, along with water additives, effectively reduces stress and enhances survival of *Labeo rohita* fingerlings during transport (Biswal et al., 2020). Optimal stocking densities minimize stress, enhance post-transport survival, and reduce disease risk, while multiple stressors like poor water quality and high density collectively weaken fish health (Suguna, 2020). Conversely, adverse conditions elevate cortisol and suppress immunity, increasing infection risk and reducing growth (Duran and Çenesiz, 2023; Ahmed and Shenoy, 2012). Several studies have examined density effects on fingerling survival across species (Honnananda et al., 2021; Lima et al., 2020; Xavier et al., 2018), but most cover short durations (<4 h), unlike India's commercial transport lasting 6–12 h or more. Few address delayed mortality during 24–48 h recovery, often linked to sublethal stress, osmotic imbalance, and tissue injury (Noga, 2010). Rohu shows similar post-transport vulnerability. Farmer awareness of stocking density varies; commercial farmers follow guidelines, but smallholders rely on local advice, often causing higher mortality. Awareness is improving through training, hatchery demonstrations, and government initiatives. Optimizing density for transport duration is vital. Regional climate and temperature variations add complexity, highlighting the need for context-based studies. However, integrated research on transport duration and density in IMCs is still limited. Such studies are key for sustainable aquaculture, addressing overlooked stress factors and supporting welfare-focused, low-antibiotic systems like recirculating and oxygen-controlled units. Hence, this study aimed to evaluate the effects of varied packing densities during 12 and 24 h transportation of Rohu fingerlings, analyzing physiological and hematological responses under differential hapa rearing for two weeks.

2. MATERIALS AND METHODS

The study was conducted from September–October, 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.

2.1. Experimental design

The study was conducted to assess the effects of transport duration and packing density on the physiological and hematological responses of Rohu (*Labeo rohita*) fingerlings. In this study, two transport durations, 12 h and 24 h, were tested under five varied packing densities. The study comprised five and seven groups in the 12 h and 24 h transportation trials, respectively. In the 12 h transport, Groups 1–5 were stocked at 4, 6, 8, 10, and 12 fingerlings l^{-1}, while in the 24 h transport, Groups 1–7 were stocked at 1, 2, 3, 4, 5, 6, and 7 fingerlings l^{-1}, respectively. Post-transport stress responses were monitored by transferring a required number of transported fingerlings to hapa for rearing at a uniform stocking density (20 fish m^{-2}) for 24 h, 48 h, 1 week, and 2 weeks, with groups and replications maintained as per the transport design. Physiological and hematological parameters were recorded at each stage to evaluate stress recovery. Finally, based on the observations, an optimum stocking density was suggested to achieve healthy and maximum survival in fingerlings. This facilitated systematic understanding and critical insights into how density and duration interact to affect fish health, survival, and stress recovery. A schematic representation of the experimental

design was shown in Figure 1.

Figure 1: Schematic of the experimental design showing effects of transit duration and packing density on Rohu fingerlings (80–100 mm). Two transport durations (12 h: 5 packing densities; 24 h: 7 packing densities) were tested. Post-transport, fish were held in hapas (20 fish m^{-2}) for 2 weeks with sampling at 24 h, 48 h, 1 week, and 2 weeks. Metabolic, hematological, physicochemical, and physical parameters were analyzed to assess stress and recovery.

2.2. Source of fish fingerlings, conditioning, and packing

The fry of rohu (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. After 30 days of rearing, the fingerlings underwent a pre-transport conditioning period of approximately 19.8 h. The selected period also corresponds with the standard 24-h fasting duration frequently reported for *Labeo rohita*, ensuring

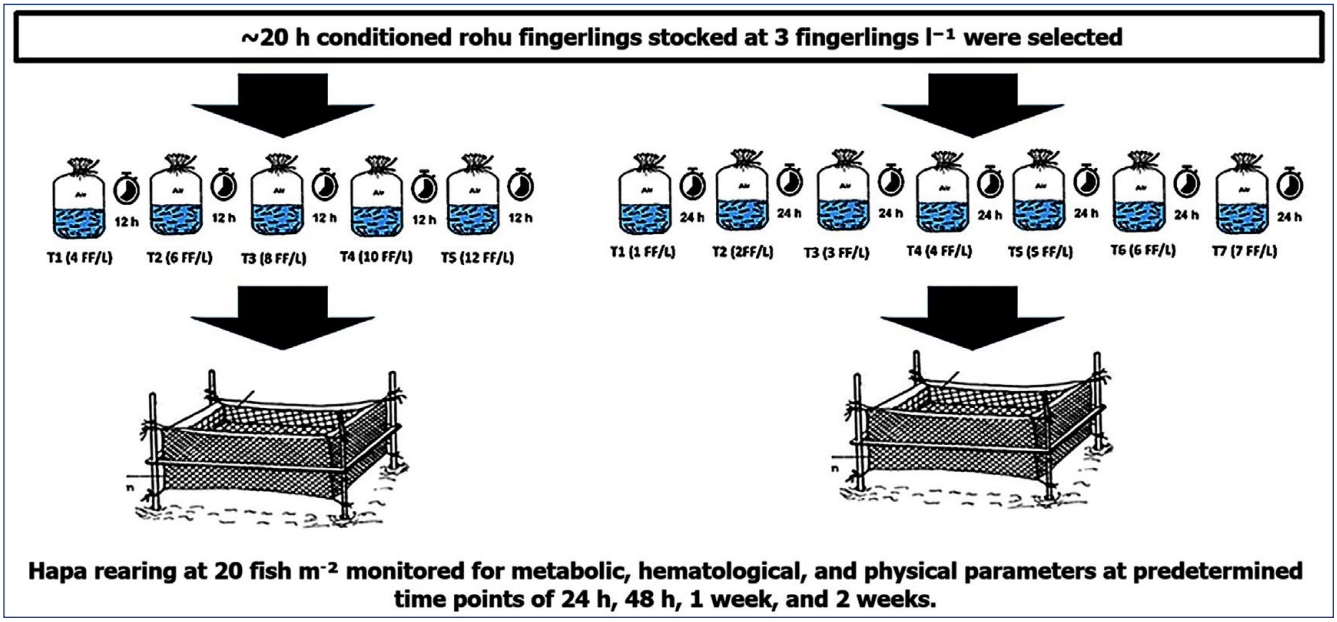

Figure 1: Schematic of the experimental design showing effects of transit duration and packing density on Rohu fingerlings (80–100 mm). Two transport durations (12 h: 5 packing densities; 24 h: 7 packing densities) were tested. Post-transport, fish were held in hapas (20 fish m^{-2}) for 2 weeks with sampling at 24 h, 48 h, 1 week, and 2 weeks. Metabolic, hematological, physicochemical, and physical parameters were analyzed to assess stress and recovery

effective gut clearance while minimizing both metabolic waste and transport-induced stress Chatterjee et al., 2010; Goswami et al., 2020; Jia et al., 2018). Fingerlings were packed in oxygenated polythene bags of 30 l capacity, containing one-third water. Prior to packing, samples from selected fingerlings for experiment were maintained in hapa for two weeks to analyze baseline stress indicators and water parameters.

2.3. Evaluation of stress indicators

Prior to blood sampling, fish were anesthetized using clove oil at a concentration of 50 µl L^{-1} to minimize handling stress. Blood was collected via caudal venipuncture using heparinized syringes and immediately transferred into EDTA-coated plastic tubes to prevent coagulation. The collected blood samples were used for analysis of the stress indicators, for glucose, lactate, hemoglobin, and hematocrit levels, as per the standard protocols to assess physiological and hematological responses. Blood glucose levels were measured using a OneTouch Ultra-Glucose Meter (Bartonková et al., 2017). Hemoglobin concentration was estimated using the cyanmethemoglobin method with Drabkin's reagent (#23RR621-80, ARKAY Healthcare Pvt. Ltd, Surat, Gujrat, India), following the protocol described previously (Witeska et al., 2022). Briefly, 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 of the samples with those of a standard cyanmethemoglobin solution. Glycogen estimation was performed using fish muscles. Here, the fish were anesthetized 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 with anthrone reagent (Nowlan et al., 2011). Hematocrit levels were determined by using a microhematocrit centrifuge (Blaxhall and Daisley, 1973). Additionally, blood lactate levels were measured using a commercially available Lacto Spark portable lactate meter (Sensa Core Medical Instrumentation Pvt. Ltd., Telangana, India) by following the methodologies described earlier (Brown et al., 2008; Wells and Pankhurst, 1999). All the physiological stress indicators were measured after 12 h, 24 h of transportation and subsequent hapa rearing 24 h, 48 h, 1 week, and 2 weeks from the transportation day.

2.4. Evaluation of survival and physically stress

Post-transport, fingerlings were assessed for physical stress indicators and survival. Weak individuals were identified by signs of lethargy, diminished response to external stimuli, and erratic or sluggish swimming behavior. Isolated fingerlings displayed irregular or uncoordinated movements, distinct from the cohesive motion of the group. Injured fish were recognized by visible external lesions, including fin damage, hemorrhages, or abnormal body coloration. Furthermore, live fingerlings were counted during transfer to the hapa post-transport and compared with the initial number recorded prior to transport. Survival or mortality percentages were then calculated using standard calculations.

2.5. Statistical analysis

All statistical calculations and relevant analyses were performed using SPSS v16 (Chicago, IL, USA). Data is shown as the mean±SD. A one-way analysis of variance (ANOVA) with Tukey's post-hoc test was used to make multiple comparisons. $p<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

This study highlighted the critical role of packing density in influencing post-transport survival and the need to optimize transport protocols for healthy fingerlings. It also emphasized delayed mortality, as survival declined during two weeks of rearing at higher densities, indicating cumulative stress and crowding. Therefore, both immediate and delayed mortality should be addressed in transport management. The Indian subcontinent, with highly variable climatic conditions, infrastructure, and aquaculture practices, makes fingerling transportation crucial to industry success. Fingerlings tolerate longer durations (8–24 h) with lower oxygen demand and reduced ammonia toxicity (Bui et al., 2013). Recent studies showed rohu fry could be safely transported for 8 h with <4% mortality (Das et al., 2025). However, long-distance transport remained necessary due to uneven aquaculture resource distribution. Many Indian farmers overlook optimal packing density, emphasizing quantity over quality, which elevated stress, reduced survival, and caused unrecognized financial losses. Thus, increasing awareness and promoting evidence-based density guidelines among stakeholders were vital for improving aquaculture transport efficiency.

3.1. Baseline observations on metabolic and hematological parameters

Baseline physiological and biochemical parameters of conditioned fingerlings (two weeks) were evaluated before transportation experiments. The average glucose level (31.50±1.72 mg dl^{-1}) indicated stable energy availability under non-stressful conditions. Blood glucose, a key stress biomarker, typically rose during handling and transport (Barton and Iwama, 1991); the observed value suggested a metabolically balanced state. Lactate concentration (2.78±0.21 mmol l^{-1}) reflected normal anaerobic metabolism, as elevated levels were linked to hypoxia or crowding (Wedemeyer et al., 1990). The low lactate supported efficient aerobic metabolism in hapa conditions. Glycogen reserves (248.20±2.39 µg mg^{-1}) represented a strong energy store, rapidly mobilized during stress to sustain glucose levels (Dai et al., 2024). The high value indicated adequate nutrition and energy readiness for transport stress. Hemoglobin (8.15±0.19 g dl^{-1}) and hematocrit (21.30±1.34 %) showed normal oxygen-carrying capacity. Variations in these indices were sensitive stress markers reflecting oxygen demand and overall health (Mitranescu et al., 2010; Polakof et al., 2011). Stable values confirmed the fingerlings were physiologically fit, without anemia or hemodilution. These findings provided essential baseline references to identify stress-induced variations during subsequent transportation and rearing. All baseline values were presented in Table 1.

3.2. Influence of transportation on metabolic parameters

Elevated glucose and lactate levels coupled with glycogen depletion were hallmark indicators of stress in fish, reflecting enhanced glycolytic activity and mobilization of energy reserves to cope with the transportation where hypoxic and crowded conditions were typical (Schreck, 2000). In this study, 12 h and 24 h transportation resulted in elevated glucose and lactate levels, suggesting metabolic stress

Table 1: Baseline observations on metabolic and hematological parameters prior to transportation

Parameters	Observation
	(Mean±S.D.)
Glucose (mg dl^{-1})	31.50±1.72
Lactate (mmol l^{-1})	2.78±0.21
Glycogen (µg mg^{-1})	248.20±2.39
HB (g dl^{-1})	8.15±0.19
HCT (%)	21.30±1.34

caused by handling and hypoxic conditions. Specifically, in the 12 h transport group, glucose levels increased progressively with density, peaking in T_4 (10 fingerlings l^{-1}) and T_5 (12 fingerlings l^{-1}), indicating packing density-dependent stress. After 24 h of hapa recovery (12 h+H24 h), glucose levels significantly declined (p<0.05) across all density groups, indicating partial metabolic recovery. By 48 h recovery (12h+H48 h), glucose levels stabilized near baseline values (Figure 2A). In the 24 h transport group, an apparent packing density-dependent increase in glucose was observed, similar to that of 12 h transportation. Post-transport recovery in hapa (24 h) led to a significant decrease in glucose levels (p<0.05). However, levels were still on the higher side than the baseline. Further recovery at 48 h (Figure 2B), 1-week, and 2-weeks of hapa rearing showed no significant differences among groups, indicating a plateau in recovery. Together, these observations indicated an exacerbated hyperglycemic condition due to prolonged transportation. Interestingly, the T_2 groups in both 12 h and 24 h transportation exhibited notable resilience to glucose levels. Statistically, T_2 glucose levels were not significantly different from T_1, while significantly lower than T_3, T_4, and T_5. This suggested that the T_2 stocking density might represent a threshold below which stressed responses, as indicated by hyperglycemia, remain minimal. These findings might reflect typical stress responses reported earlier (Luz and Favero, 2024; Yengkokpam et al., 2020). Specifically, the observations highlighted the heightened energy demands under stress. Here, it was believed that elevated glucose levels arose from cortisol-mediated stimulation of gluconeogenesis and glycogenolysis, which increased circulating glucose to meet the increased energy demands under stress (Barton, 2000; Barton and Iwama, 1991; Biswal et al., 2021a; Wendelaar Bonga, 1997). However, the glucose levels in T_2 (6 fingerlings l^{-1}) of the 12 h group were 135.25±3.40 mg dl^{-1}, which was significantly less than 147.50±1.29 mg dl^{-1} found in T_3 (8 fingerlings l^{-1}). These observations strongly suggested that transportation of fingerlings above 6 fingerlings l^{-1} drastically increased the metabolic stress in fingerlings. Similarly, in the 24 h group, glucose levels in T_3 (3 fingerlings l^{-1}) is 133.00±2.65 mg dl^{-1}, which was significantly lower than the 144.00±1.00 mg dl^{-1} found in T_4 (4 fingerlings l^{-1}), clearly marking the stress response between these two stocking densities. In line with these observations, lactate levels increased with packing density, peaking in T_5 (12 fingerlings l^{-1}) of the 12 h group. Following 24 h hapa recovery (12h+H24 h), lactate levels significantly decreased (p<0.05) across all groups and stabilized further at 48 h (12h+H48 h). In the 24 h transport group, lactate levels rose with density, peaking in T_7 (7 fingerlings l^{-1}). Post-transport recovery (24h+H24 h) resulted in a marked decline in lactate levels (p<0.05), with minimal change thereafter at 48 h. Interestingly, lactate levels in T_2 (6.38±0.28 mmol l^{-1}) of the 12 h group and T_3 (6.07±0.06 mmol l^{-1}) of the 24 h group were significantly lower compared to their respective higher packing density groups (T_4 and above) (Figure 2C-D). The higher lactate levels could be attributed to elevated metabolic demand and reduced oxygen availability. This stimulated glycolysis, resulting in increased lactate accumulation in plasma and tissues (Bai et al., 2024; Dai et al., 2024; R. Das et al., 2025; Wendelaar Bonga, 1997). Further, glycogen levels in both Catla and Rohu were increased gradually over time. Interestingly, absolute glycogen values observed in the present study were markedly lower than baseline levels following transportation, indicating an acute stress response triggered by crowding and handling during transportation. However, these observations appear to recover 24 h after transportation and stabilize thereafter (Figure 2E-F). This stability in glycogen levels could be attributed to uniform stocking density and better water quality parameters during hapa rearing. Collectively, these elevated metabolic parameters up to 48 h of hapa rearing suggested that recovery from transport stress was not immediate, and stabilization might require up to one week. These observations were previously noted by Pakhira et al., 2015 in Rohu. Moreover, these observations on metabolic stress indicators prompted us to focus more closely on T_2 (6 fingerlings l^{-1}) and T_3 (8 fingerlings l^{-1}) in the 12 h transport group, as well as T_3 (3 fingerlings l^{-1}) and T_4 (4 fingerlings l^{-1}) in the 24 h group. These groups exhibited distinct shifts in stress marker levels that differentiated them from both lower and higher density groups. These intermediate packing densities represented thresholds where physiological stress responses began to escalate significantly. Besides, glucose and lactate levels in these groups significantly reduced to below 50 mg dl^{-1} and 4.1 mmol l^{-1}, respectively, after 24 h and 48 h of hapa rearing. These observations fall within the normal physiological ranges for glucose (30–70 mg dl^{-1}) and lactate (2–4 mmol l^{-1}) (Sampaio and Freire, 2016) (Biswal et al., 2021a), indicating a quick recovery from transport-induced stress within 24 h. Together, these observations suggested glucose mobilization was mediated by cortisol and catecholamines and lactate accumulation resulting from anaerobic metabolism under reduced oxygen availability (Mommsen et al., 1999). Similar findings have been reported in other cyprinids and salmonids exposed to crowding or handling stress (Cho et al., 2009; Dobšíková et al., 2006; WendelaarBonga, 1997). Interestingly, the persistence of elevated metabolic parameters up to 48 h of hapa rearing suggests that recovery from transport stress was not immediate, and stabilization might require up to one week. These observations were previously noted in Rohu (Pakhira et al., 2015).

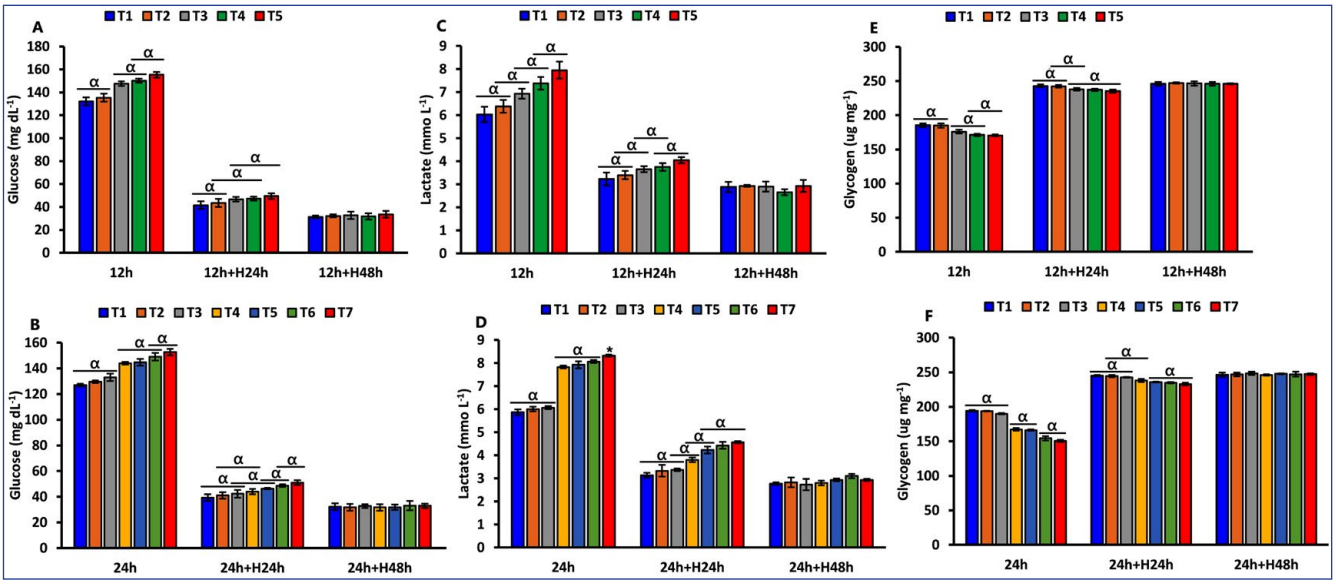

Figure 2: Impact of transportation on metabolic stress markers: (A–B) Glucose, (C–D) Lactate, and (E–F) Glycogen levels following 12 h and 24 h transportation, respectively. For 12 h transport, T_1–T_5 represent 4, 6, 8, 10, and 12 fingerlings L^{-1}; for 24 h, T_1–T_7 represent 1–7 fingerlings l^{-1}. Values are mean±S.D. α denotes non-significance (p>0.05) among treatments; groups not sharing α differ significantly (p<0.05). Absence of symbol indicates no difference; * denotes significant variation from other treatments

Table 2: Water quality under varied packing densities for 12 h transportation and subsequent hapa rearing

Packing density	pH	CO_2 (mg l^{-1})	Total alkalinity (mg l^{-1})	Total hardness (mg l^{-1})
12 h transportation				
T_1	7.28±0.17^{c}	2.25±0.50^{a}	133.50±1.29^{c}	152.50±2.08^{b}
T_2	7.25±0.13^{bc}	3.50±1.00^{ab}	131.00±1.8^{3bc}	150.75±2.22^{ab}
T_3	7.00±0.08^{ab}	3.88±0.90^{ab}	128.75±2.22^{ab}	148.50±1.91^{ab}
T_4	6.98±0.10^{a}	4.23±0.93^{b}	128.25±2.06^{ab}	147.75±1.71^{a}
T_5	6.93±0.10^{a}	4.35±0.33^{b}	126.50±1.73^{a}	146.50±2.65^{a}
Hapa rearing from above treatments group with stocking density 20 fingerlings m^{-2}				
12 h transportation+24 h hapa rearing (12 h+H24 h)				
T_1	8.00±0.32	1.25±0.50	137.25±0.96	158.00±2.45
T_2	8.00±0.29	1.50±0.58	137.00±1.41	158.50±1.29
T_3	8.03±0.26	1.75±0.50	137.25±2.22	158.00±1.83
T_4	8.03±0.22	1.50±0.58	136.75±0.96	157.25±2.22
T_5	7.95±0.24	1.50±0.58	136.50±0.58	156.75±2.22
12 h transportation+48 h hapa rearing (12 h+H48 h)				
T_1	8.10±0.18	1.75±0.50	139.00±2.94	159.25±2.22
T_2	8.05±0.24	2.25±0.96	139.00±2.94	159.25±2.63
T_3	8.03±0.26	2.00±0.82	140.25±3.59	159.75±1.71
T_4	8.10±0.29	2.00±0.82	139.25±3.30	159.50±2.38
T_5	8.08±0.22	2.25±0.96	139.75±3.59	159.25±2.63

Values expressed as mean±S.D. Within each row, values with different superscripts indicate significant differences among treatments (p≤0.05), while means sharing the same letter, or without a letter, do not differ significantly (p>0.05). Stocking densities were 4, 6, 8, 10, and 12 fingerlings l^{-1} for T_1–T_5, respectively

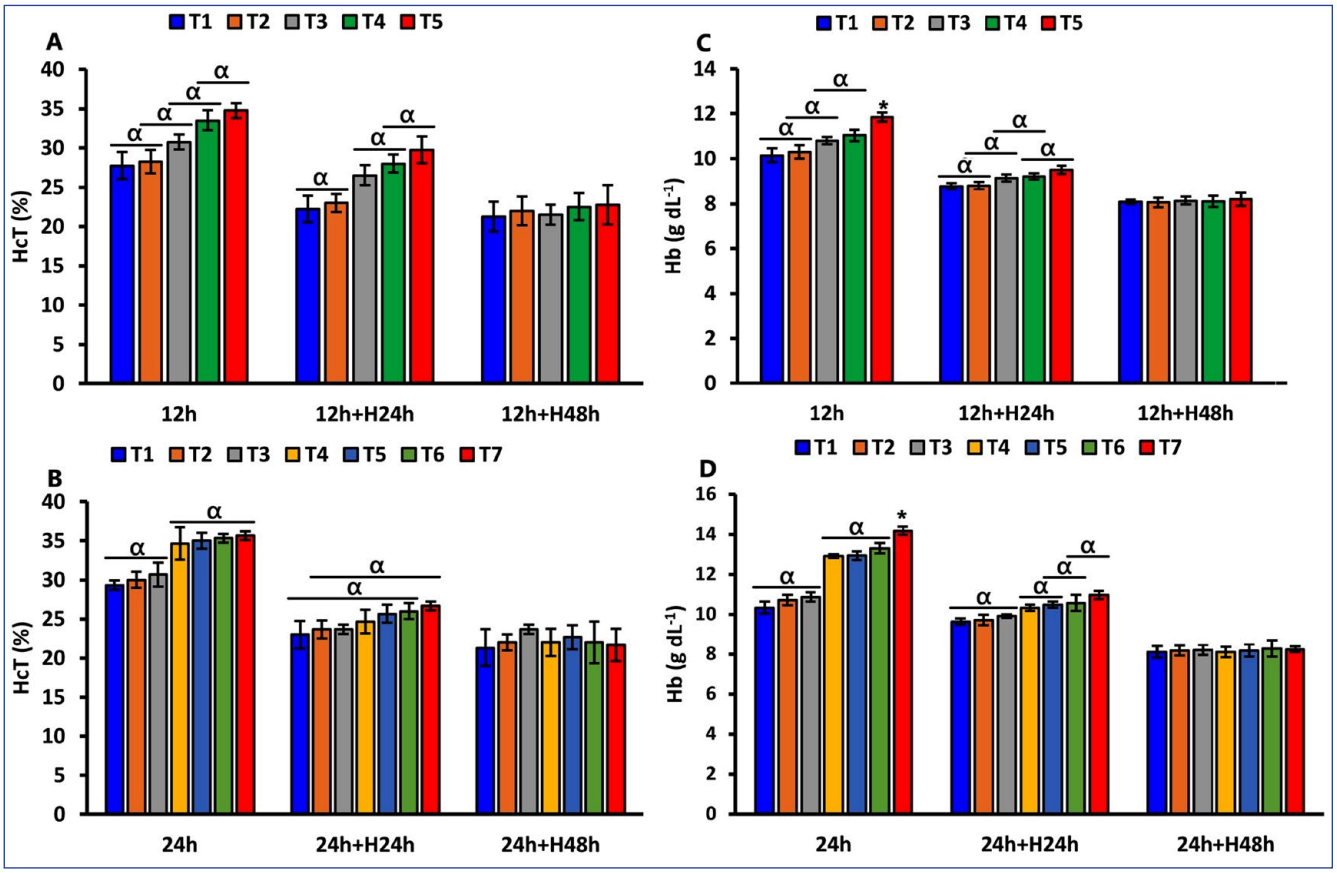

Figure 3: Impact of transportation on hematological parameters: (A–B) Hematocrit (HCT) and (C–D) Hemoglobin (HB) levels following 12 h and 24 h transportation, respectively. For 12 h transport, T_1–T_5 represent 4, 6, 8, 10, and 12 fingerlings l^{-1}; for 24 h, T_1–T_7 represent 1–7 fingerlings l^{-1}. Values are mean±S.D. α denotes non-significance (p>0.05) among treatments; groups not sharing α differ significantly (p<0.05). Absence of symbol indicates no difference; * denotes significant variation from other treatments

3.3. Influence of transportation on hematological parameters

Generally, normal hematological ranged in IMCs were HB 8.5–11.6 g dl^{-1} and HCT 28.3–38.6% Das, et al., 2006; Malathi et al., 2021). HCT serve as sensitive biomarkers for assessing the physiological condition and stress status of fish during transportation (Abdel-Tawwab et al., 2019; Das et al., 2006). In this study, HCT levels followed a similar trend, increasing with density, with the highest values in T_4 and T_5, and gradually normalizing during recovery. In the 24 h transport group, HCT levels rose with density, peaking in T_6 (6 fingerlings l^{-1}) and T_7 (7 fingerlings l^{-1}). Post-transport recovery (24h+H24 h) resulted in a marked decline in HCT levels compared to the 24 h group (p<0.05), with minimal change thereafter at 48 h (Figure 3A-B). Further, recovery over 1 and 2-weeks found to be similar as 48 h hapa rearing observations. Similarly, at 12 h post-transport, Rohu fingerlings in higher packing density groups showed elevated HB levels, reaching 11.85±0.21 g dl^{-1} (T_5) and exceeding the reference range. Following 24 h of hapa rearing, HB levels decreased and restored to the normal range. Later, following 48 h of hapa rearing, HB levels further reduced and maintained between 8.0–8.2 g dl^{-1} (Figure 3C-D). Recovery over the 48 h hapa rearing period following transportation facilitated a progressive normalization of HCT values. This decline from elevated post-transport levels indicated restoration of erythrocyte balance and re-establishment of oxygen-carrying capacity, reflecting the alleviation of transport-induced hypoxia and stress (Gupta and Bharat, 2010). The trend highlights the effectiveness of post-transport acclimation in mitigating hematological disturbances.

3.4. Transport-Induced variations in water quality

Temperature, dissolved oxygen (DO), and total ammonia nitrogen (TAN) were key water quality parameters influencing fish health during transportation. Optimal temperature maintained metabolic stability, adequate DO prevented hypoxia, and proper packing density regulates TAN levels. During both 12 h and 24 h transportation, water temperature was significantly higher than post-transport rearing conditions across all densities. This rise

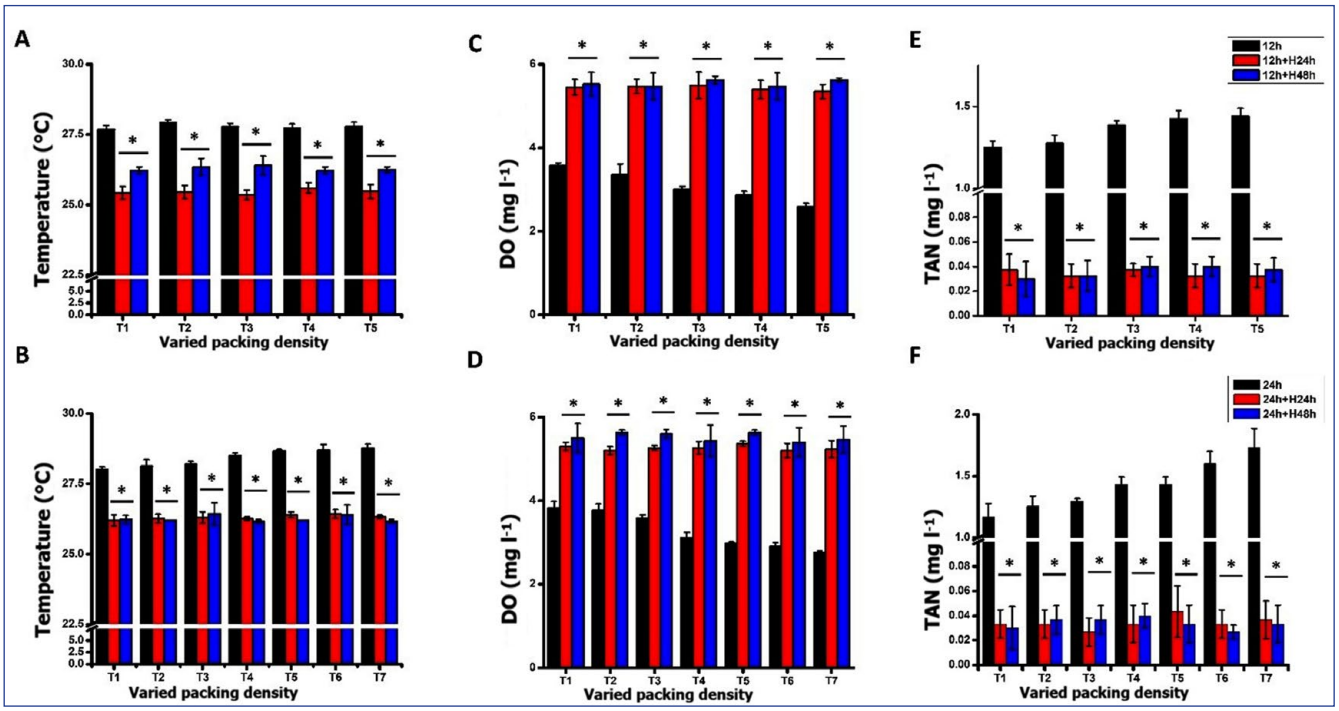

Figure 4: Transportation-induced variations in water quality parameters. For 12 h transport, T_1–T_5 represent 4, 6, 8, 10, and 12 fingerlings l^{-1}; for 24 h transport, T_1–T_7 represent 1–7 fingerlings l^{-1}. Post-transport recovery was evaluated in hapa at 24 h (H24 h) and 48 h (H48 h) (H=hapa rearing). Values are mean±S.D. A difference of $p<0.05$ was considered significant. *Denotes values significantly different from the 12 h group. TAN: Total ammonia nitrogen

likely resulted from restricted heat dissipation in containers or bags, absence of active cooling in small-scale operations, and collective metabolic heat from fish (Caspers, 1983; Wendelaar Bonga, 1997; Luz and Favero, 2024). After transfer to hapa, temperature declined to 25–26°C and remained stable during the two-week rearing (Figure 4A–B).

DO levels dropped markedly during transportation ($p<0.05$), especially at higher densities, indicating oxygen depletion from respiration. DO in T_2 (3.36±0.25 mg l^{-1}) and T_3 (3.01±0.06 mg l^{-1}) of the 12 h group approached the critical threshold (<3.0 mg l^{-1}) (Branson and Southgate, 2008; Turnbull et al., 2008), suggesting severe hypoxia. During hapa recovery, DO levels exceeded 5 mg l^{-1} in all groups (Figure 4C–D), showing that controlled hapa conditions effectively reversed hypoxia. Similar recovery trends were reported earlier, where pre-release hapa conditioning improved fish physiological responses (Carneiro and Urbinati, 2002; Chatterjee et al., 2006; Das et al., 2015; Honnananda et al., 2020).

TAN concentrations increased with packing density, peaking in the densest groups (T_5 in 12 h and T_7 in 24 h). Post-transportation and hapa rearing differences were significant ($p<0.05$), confirming recovery (Figure 4E–F). One-and two-week hapa rearing showed stabilization similar to 24–48 h recovery, indicating normalization with ambient water and climate. Other parameters, including CO_2, pH, alkalinity, and hardness, also reflected transport stress. CO_2 rose with density, lowering pH below 7 in high-density groups. Hapa recovery stabilized CO_2 (1.5–2.2 mg l^{-1}) and restored pH, signifying improved water quality. In contrast, alkalinity and hardness declined immediately post-transport but stabilized during recovery, alkalinity (136–140 mg l^{-1}) and hardness (157–160 mg l^{-1}) compared to >167 mg l^{-1} during transport. All water quality parameters are summarized in Tables 2 and 3. Previous studies confirm that higher density increases ammonia due to elevated metabolism and waste output (Honnananda et al., 2020; Saether et al., 2016).

Overall, transportation significantly affected temperature, DO, and TAN, emphasizing the importance of post transport recovery for alleviating stress and ensuring healthy fingerlings before grow-out stocking.

3.5. Influence of transportation stress on survival

The study on varied packing densities clearly demonstrated the influence of transportation and density on fingerling survival. In the 12 h transport group, mortality increased progressively with density. T_1 (4 fingerlings l^{-1}) and T_2 (6 fingerlings l^{-1}) exhibited the lowest mortality (<5%), whereas T_4 (10 fingerlings l^{-1}) and T_5 (12 fingerlings l^{-1}) showed

Table 3: Water quality under varied packing densities for 24 h transportation and subsequent hapa rearing

Packing density	pH	CO_2 (mg l^{-1})	Total Alkalinity (mg l^{-1})	Total hardness (mg l^{-1})
24 h transportation				
T_1	7.33±0.06^{d}	1.33±0.58^{a}	133.00±2.65^{b}	152.67±2.52^{c}
T_2	7.17±0.06^{cd}	1.67±0.58^{a}	132.33±2.52^{b}	149.33±1.15^{bc}
T_3	7.10±0.10^{bcd}	2.00±1.00^{ab}	130.00±2.00^{ab}	147.33±2.08^{ab}
T_4	7.00±0.10^{bc}	2.67±0.58^{abc}	127.67±2.08^{ab}	147.67±0.58^{ab}
T_5	6.97±0.12^{abc}	3.30±0.10^{bc}	127.00±2.65^{ab}	146.33±1.53^{ab}
T_6	6.87±0.12^{ab}	3.73±0.06^{c}	124.67±1.15^{a}	144.00±2.65^{a}
T_7	6.73±0.06^{a}	3.83±0.06^{c}	124.33±2.31^{a}	144.33±0.58^{a}
Hapa rearing from above treatments group with stocking density 20 fingerlings m^{-2}				
24 h transportation+24 h hapa rearing (24 h+H24 h)				
T_1	8.17±0.06	2.03±0.06	137.00±2.65	154.00±1.00
T_2	8.23±0.21	2.03±0.06	137.00±2.65	154.00±1.00
T_3	8.17±0.29	2.10±0.17	136.33±1.53	155.00±2.00
T_4	8.20±0.17	2.10±0.10	135.67±0.58	154.67±1.53
T_5	8.30±0.20	2.03±0.06	137.67±2.08	155.33±0.58
T_6	8.33±0.06	2.07±0.12	136.00±1.00	154.33±1.15
T_7	8.30±0.00	2.10±0.17	137.00±1.00	155.33±0.58
24 h transportation+48h hapa rearing (24 h+H48 h)				
T_1	7.93±0.15	1.67±0.58	139.00±3.61	160.00±2.00
T_2	8.10±0.26	2.00±1.00	138.67±3.51	158.67±2.89
T_3	8.07±0.23	2.00±1.00	138.33±3.06	159.00±2.65
T_4	7.97±0.21	1.67±0.58	138.00±2.65	159.00±2.65
T_5	8.10±0.26	2.00±1.00	138.67±3.51	158.67±2.89
T_6	8.03±0.31	2.67±0.58	142.00±2.00	158.33±2.31
T_7	8.03±0.21	1.67±0.58	138.67±3.51	159.00±2.65

Values expressed as mean±S.D. Within each row, values with different superscripts indicate significant differences among treatments ($p \leq 0.05$), while means sharing the same letter, or without a letter, do not differ significantly ($p > 0.05$). Stocking densities were 4, 6, 8, 10, and 12 fingerlings l^{-1} for T_1–T_7, respectively

significantly higher mortality (>10%, $p<0.05$) compared to T_1 and T_2 (Figure 5A). Moreover, T_3–T_5 groups displayed a higher proportion of weakened and injured fingerlings (Table 4), corresponding with increased mortality and indicating reduced physical resilience under crowding stress. Following 24 h of hapa rearing (24h+H24 h), mortality declined across all groups. However, delayed mortality became evident after 48 h of hapa rearing, particularly in T_3–T_5, where mortality rose from ~2.5% to over 15%, suggesting post-transport injury effects (Figure 5B). Subsequent hapa rearing further reduced mortality, and no mortality were recorded after the first and second weeks in both transport group. A similar trend was observed in fingerlings transported for 24 h and during subsequent hapa rearing (Table 5).

Survival outcomes in this study highlighted the strong influence of transport duration and packing density on post-transport viability. In 12-h transport, T_2 (6 fingerlings l^{-1}) maintained high survival, while higher densities (8–12 fingerlings l^{-1}) showed sharp declines, and in 24-h transport, T_3 (3 fingerlings l^{-1}) provided the best long-term survival (Table 6), confirming that intermediate densities were optimal for minimizing stress and ensuring transport efficiency. This was consistent with previous fish studies reporting that rising density and duration increase mortality, compromise physiological and immune functions, and that lower densities, although favorable for survival, reduced transport efficiency, whereas intermediate densities helped maintain water quality and survival (Bai et al., 2024; Liu et al., 2022; Wang et al., 2024).

Table 4: Weak, isolated, and injured fingerlings across treatments after 12 h transportation and subsequent hapa rearing

Varied density	% Weak	% Isolated	% Injured
12 h transportation			
T_1	13.54±3.99^{a}	2.08±2.41^{a}	2.08±2.41^{a}
T_2	15.28±1.60^{ab}	4.86±2.66^{ab}	2.78±2.27^{ab}
T_3	20.83±1.70^{bc}	5.21±2.08^{ab}	3.65±1.99^{ab}
T_4	23.75±2.85^{c}	6.67±1.36^{b}	6.25±1.60^{ab}
T_5	26.74±3.08^{c}	7.99±1.33^{b}	7.64±2.41^{b}
Hapa rearing from above treatments group with stocking density 20 fingerlings m^{-2}			
12 h transportation+24 h hapa rearing (12 h+H24 h)			
T_1	5.00±5.77	NIL	NIL
T_2	7.50±5.00	NIL	NIL
T_3	12.50±5.00	NIL	NIL
T_4	15.00±5.77	NIL	NIL
T_5	17.50±9.57	NIL	NIL
12 h transportation+48 h hapa rearing (12 h+H48 h)			
T_1	NIL	NIL	NIL
T_2	NIL	NIL	NIL
T_3	NIL	NIL	NIL
T_4	NIL	NIL	NIL
T_5	NIL	NIL	NIL
12 h transportation+1 week and 12 h transportation+2 week			
T_1–T_5	NIL	NIL	NIL

Values expressed as mean±S.D. Within each row, values with different superscripts indicate significant differences among treatments ($p \leq 0.05$), while means sharing the same letter, or without a letter, do not differ significantly ($p > 0.05$). Stocking densities were 4, 6, 8, 10, and 12 fingerlings l^{-1} for T_1–T_5, respectively

3.6. Transportation stress and delayed mortality

Transportation duration and packing density significantly affected the physical condition of Rohu fingerlings. After 12 h transport, weak fish ranged from 13–15% in low-density groups (T_1–T_2) to 20–27% in high-density groups (T_3–T_5). Hapa recovery reduced weak fish, with faster recovery at lower densities (<12.5%), while higher densities (>15%) showed incomplete recovery. After 24 h transport, weak fish ranged widely, 0–27%, indicating prolonged stress. None of the fish in T_1–T_3 were weak, but in T_4–T_7, they increased with packing density. Interestingly, following 12 h and 24 h transport, the fingerlings were injured, but the injury rate increased with the packing density. It was clear that crowding disrupts normal fish movement, often causing erratic behavior that could result in physical injury. Also, observations were consistent with earlier studies indicating that crowding intensifies competition for oxygen and increased metabolic waste, thereby accelerating stress (Cox and Felder, 2025). Furthermore, mortality rates in the

Table 5: Weak, Isolated, and Injured fingerlings across Treatments after 24 h transportation and subsequent hapa rearing

Varied density	% Weak	% Isolated	% Injured
24 h transportation			
T_1	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
T_2	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
T_3	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
T_4	15.28±2.41^{b}	4.17±0.00^{b}	1.39±2.41^{ab}
T_5	24.44±3.85^{c}	8.89±1.92^{b}	2.22±1.92^{ab}
T_6	25.00±2.78^{c}	9.26±1.60^{b}	2.78±0.00^{ab}
T_7	27.78±3.64^{c}	9.52±2.38^{b}	4.76±2.38^{b}
Hapa rearing from above treatments group with stocking density 20 fingerlings m^{-2}			
24 h transportation+24 h hapa rearing (24 h+H24 h)			
T_1	0.00±0.00^{a}	0.00±0.00^{a}	NIL
T_2	0.00±0.00^{a}	0.00±0.00^{a}	NIL
T_3	3.33±5.77^{ab}	0.00±0.00^{a}	NIL
T_4	6.67±5.77^{abc}	3.33±5.77^{ab}	NIL
T_5	10.00±0.00^{abc}	6.67±5.77^{ab}	NIL
T_6	13.33±5.77^{bc}	10.00±0.00^{ab}	NIL
T_7	16.67±5.77^{c}	13.33±5.77^{b}	NIL
24 h transportation+48 h hapa rearing (24 h+H48 h)			
T_1	0.00±0.00	NIL	NIL
T_2	0.00±0.00	NIL	NIL
T_3	0.00±0.00	NIL	NIL
T_4	3.70±6.42	NIL	NIL
T_5	3.70±6.42	NIL	NIL
T_6	7.41±6.42	NIL	NIL
T_7	11.11±0.00	NIL	NIL
24 h transportation+1 week and 24 h transportation+2 week			
T_1–T_7	NIL	NIL	NIL

Values expressed as mean±S.D. Within each row, values with different superscripts indicate significant differences among treatments ($p \leq 0.05$), while means sharing the same letter, or without a letter, do not differ significantly ($p > 0.05$). Stocking densities were 4, 6, 8, 10, and 12 fingerlings l^{-1} for T_1–T_7, respectively

T_3, T_4, and T_5 groups showed a significant rise after 48 h of hapa rearing, increasing from ~2.5% to >15%, strongly suggesting a trend of delayed mortality. This trend indicated that despite fish seeming to recover post-transport, sublethal stress, tissue injury, or immune suppression might ultimately lead to mortality after a lag period. All the observations on weak, injured, and isolated fingerlings are shown in Table 4, 5 and Figure 5. Additionally, survival of fingerlings was influenced by both transportation duration and packing density. After 12 h transport, survival was highest in T_1 (95.83%) and T_2 (95.14%), but declined progressively with density, reaching 85.42% in T_5. Following hapa rearing, survival further decreased, most notably in higher densities (77.50% in T_5). After 24 h transport, survival again declined with density, from 94.44% (T_1) to 71.43% (T_7). Hapa rearing partially stabilized survival, but reductions persisted, especially in high-density groups (66.67–70%). All the observations on survival were shown in Table 6. These observations suggested the possibility of delayed mortality.

The observations of the investigation marked a substantial advancement beyond most previous studies, which generally emphasized durations of under four hours. This extended post-transport observation of mortality provided distinctive insights into delayed death events that were frequently overlooked in short-term studies. Also, these delayed observations could be associated with osmotic imbalance, metabolic exhaustion, and compromised epithelial integrity following handling and transport stress. This type of mortality was referred to as delayed mortality syndrome (DMS) (Noga, 2010). Importantly, stress-induced changes in plasma osmolarity, a decrease in freshwater species, and an increase in saltwater species were hallmarks of DMS (Robertson et al., 1988). Weight losses of up to 10% have been observed within 9–49 h post-transport. This reduced weight was attributed to dehydration driven by osmotic dysfunction (Sleet and Weber, 1982). However, it appeared that DMS was more pronounced in marine fish rather than freshwater fish. Together, these results highlighted the critical influence of conditioning of fingerlings prior to transportation and transport packing density on fish survival post-transportation. In line with these findings, Das et al., 2025 observed density-dependent delayed mortality in *Labeo rohita* and Catla catla fry subjected to high-density transport. Similar patterns have been described in Nile tilapia (Oreochromis niloticus), where fingerlings transported for 6 h at high densities showed low immediate mortality but elevated post-transport mortality within 24–72 h (Ahmed et al., 2016). Likewise, Wedemeyer, 1996 noted that salmonids often exhibit stress-induced delayed losses after hauling and Refaey and Li,

Table 6: Survival percentage after 12/24-hour transportation and at 2-week of hapa rearing

Varied density	12 h		24 h	
	After transportation	After Hapa rearing	After transportation	After Hapa rearing
T_1	95.83±3.40^{c}	95.00±5.77^{c}	94.44±9.62^{c}	93.33±5.77^{d}
T_2	95.14±1.39^{bc}	92.50±5.00^{bc}	91.67±0.00^{bc}	90.00±0.00^{cd}
T_3	89.58±1.70^{ab}	82.50±5.00^{ab}	90.74±3.21^{abc}	86.67±5.77^{bcd}
T_4	88.33±1.36^{ab}	80.00±0.00^{a}	83.33±0.00^{ab}	80.00±0.00^{abc}
T_5	85.42±0.80^{a}	77.50±5.00^{a}	82.22±1.92^{ab}	76.67±5.77^{ab}
T_6	NA	NA	72.22±0.00^{ab}	73.33±5.77^{a}
T_7	NA	NA	71.43±0.00^{a}	70.00±0.00^{a}

NA, not applicable. Values were expressed as mean±S.D. Within each row, values with different superscripts indicate significant differences among treatments ($p \leq 0.05$), while means sharing the same letter do not differ significantly ($p > 0.05$). Stocking densities were 4, 6, 8, 10, and 12 fingerlings L^{-1} (T_1–T_5) and 1–7 fingerlings L^{-1} (T_1–T_7) for the 12 h and 24 h transport groups, respectively

2018 reported that channel catfish (Ictalurus punctatus) experienced post-transport mortalities within 2–4 days due to immunosuppression. Together, these results highlighted that delayed mortality was not unique to *Labeo rohita* but represented a widespread transport-related challenge in aquaculture species, emphasizing the critical influence of pre-conditioning, transport density, and recovery conditions on post-transport survival.

Collectively, the comprehensive analysis of metabolic stress markers, physiological condition, survival rates, and water quality parameters indicated that a packing density of 6 fingerlings l^{-1} was optimal for short-distance transportation of up to 12 h. This helps ensured that fingerlings experience minimal stress, leading to high survival rates following transportation. In contrast, for long-distance transportation (~24 h), a lower density of 3 fingerlings l^{-1} proved more suitable. This density minimized physiological stress and post-transport mortality while maintaining critical

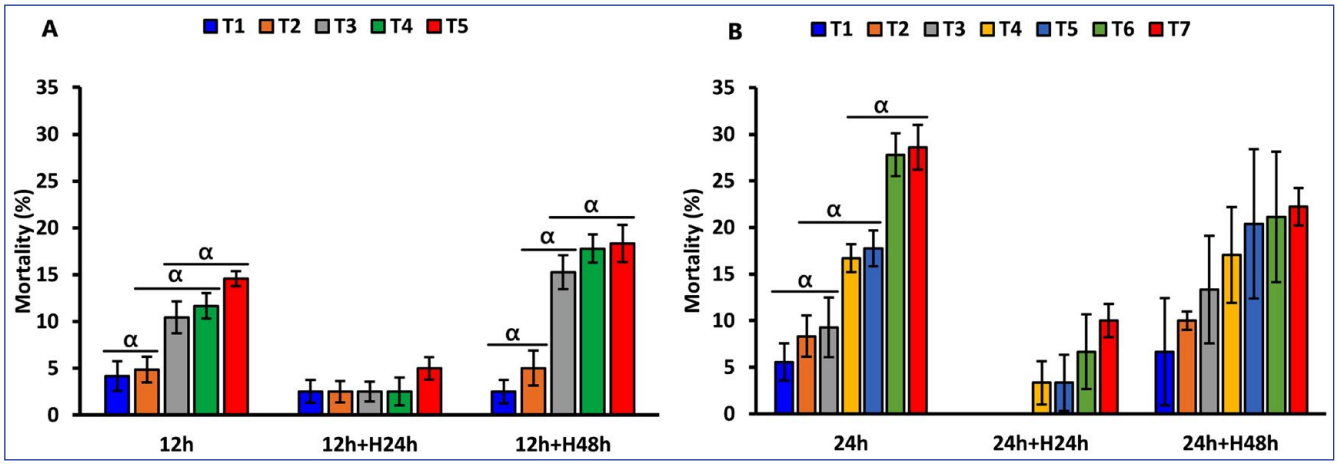

Figure 5: Mortality after transportation and subsequent hapa rearing. For 12 h transport (A), T_1–T_5 represented 4, 6, 8, 10, and 12 fingerlings l^{-1}; for 24 h transport (B), T_1–T_7 represent 1–7 fingerlings L^{-1}. Post-transport recovery was evaluated in hapa at 24 h (H24 h) and 48 h (H48 h) (H=hapa rearing). Values were mean±S.D. α denoted non-significance (p>0.05) among treatments; groups not sharing α differed significantly (p<0.05). Absence of symbol indicated no difference; * denoted significant variation from other treatments

water parameters within acceptable limits, making it the recommended threshold for extended transport durations. However, as the study focused on a single species, *Labeo rohita*, the findings might not be directly transferable to other fish species that exhibited different stress tolerances or physiological responses to transportation, which represented a limitation of the study. In addition, the study did not include cortisol measurement, a primary endocrine indicator of stress in fish that mediated key secondary responses such as hyperglycemia, ion imbalance, and immunosuppression. Although secondary markers like glucose, lactate, and hematocrit were assessed, the absence of direct cortisol analysis limits a complete understanding of the endocrine basis of transport stress. Future research should integrate cortisol quantification with metabolic and hematological indices to provide a more comprehensive evaluation of stress physiology during fingerling transportation. Despite the limitation, there was a positive note on the study. The study included a two-week post-transport observation period. This marks a substantial advancement beyond most previous studies, which generally emphasized durations of under four hours. This prolonged surveillance yields distinctive insights regarding deferred physiological effects and mortality that were often overlooked. Here, conclusions were based on the prolonged surveillance, which captured delayed manifestations of stress responses, physiological imbalances, and mortality events that would otherwise remain undetected. Therefore, the present investigation provided a more comprehensive understanding of the cumulative impacts of transport stress on rohu fingerlings, thereby offering critical insights for developing evidence-based recommendations on optimal packing density and transport management practices. Therefore, comprehensive investigations similar to the study involving multiple fish species were essential, especially those with transport additives such as sedatives, buffers, and antioxidants, to evaluate their effectiveness in mitigating stress responses during transportation.

4. CONCLUSION

Transport duration and packing density significantly influence physiological stress responses, water quality, and survival of *Labeo rohita* fingerlings. Elevated glucose, lactate, and hematocrit levels, alongside deviations in DO and TAN, indicated cumulative stress from higher density and longer transport. A density of 6 fingerlings l^{-1} was optimal for 12 h, while 3 fingerlings l^{-1} for 24 h transportation. Observations from a two-week post-transport period, revealed delayed stress and mortality, suggesting the need for species-specific transport standardization.

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