

ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 Indexing: Embase
 IJABR 2026; 10(9): 871-875
www.biochemjournal.com
 Received: 26-06-2026
 Accepted: 09-08-2026
 Published: 28-09-2026

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Effect of water quality parameters in the transportation of Rosy Barb (*Pethia conchonius*) with eugenol encapsulated beads

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9k.10204>

ABSTRACT

The rosy barb (*Pethia conchonius*) is among the most widely traded freshwater ornamental fish species, and its live transportation is frequently accompanied by progressive deterioration of transport-water quality. This study evaluated the effect of five treatments; T_1 (control), T_2 (Eugenol Sol.), T_3 (1% CaCl_2), T_4 (2% CaCl_2) and T_5 (5% CaCl_2) on temperature, dissolved oxygen (DO), free carbon dioxide (CO_2), pH and total ammonia over a simulated 24-hour closed-system transport of rosy barb fingerlings. Water quality was recorded at the initial (0 h), intermediate (12 h) and final (24 h) sampling points. Temperature increased steadily and comparably across all treatments ($22.3\text{--}23.0^\circ\text{C}$ initially to $25.0\text{--}26.0^\circ\text{C}$ at 24 h), indicating that the additives had no measurable influence on thermal build-up. In contrast, DO, CO_2 , pH and total ammonia diverged significantly among treatments as exposure time progressed. Fish held T_4 retained the highest dissolved oxygen (5.33 ± 0.28 mg/L) and the lowest carbon dioxide (11.33 ± 0.28 mg/L) and total ammonia (0.034 ± 0.003 mg/L) at 24 h, together with the smallest pH decline (5.70 ± 0.17), whereas the untreated control (T_1) showed the most severe deterioration on every parameter (DO 1.56 ± 0.40 mg/L; CO_2 15.76 ± 0.25 mg/L; ammonia 0.108 ± 0.003 mg/L; pH 4.06 ± 0.05). T_3 and T_5 produced intermediate, statistically distinct improvements over the control, while T_2 offered only marginal protection. These results indicate that mild sedation with T_4 , by suppressing metabolic rate and hence oxygen consumption and nitrogenous waste output, is the most effective single-additive strategy among those tested for maintaining favourable water quality during the transportation of rosy barb, followed by T_3 . The findings provide practical guidance for hatchery operators and ornamental fish exporters seeking to reduce transport-related stress and mortality in this species.

Keywords: Rosy barb, *Pethia conchonius*, Live fish transportation, Ornamental fish, Encapsulated beads, Eugenol

1. Introduction

Live fish transportation is a critical component of modern aquaculture, essential for moving fish across all life stages between facilities. However, extended transport exposes fish to severe stressors like repeated handling, confinement, high stocking densities, and deteriorating water quality. These conditions compromise animal welfare, impair post-transport performance, and increase mortality (Berka, 1986; Wedemeyer, 1996; Harmon, 2009) [1, 2, 3]. Transportation-related stress stimulates the hypothalamic-pituitary-interrenal (HPI) axis, resulting in increased cortisol secretion and a cascade of metabolic and physiological responses. These responses may include hyperglycaemia, elevation of metabolic activity and oxygen demand, increased ammonia excretion, and enhanced oxidative stress (Barton *et al.*, 1991; Wendelaar Bonga, 1997) [4, 5]. At the same time, depletion of dissolved oxygen and the accumulation of carbon dioxide and ammonia within the transport medium can intensify physiological stress and adversely affect fish survival, particularly during extended transportation periods (Colt, 2006) [6]. Therefore, the development of strategies that can reduce transportation-associated stress while preserving suitable water-quality conditions represents an important challenge in live fish transport.

The application of sedative or anaesthetic agents during transportation has been widely recognized as a potential method for mitigating stress by suppressing excessive swimming activity, lowering metabolic demands and oxygen consumption, and reducing physical injuries associated with handling and confinement. Such effects can contribute to improved fish welfare and facilitate recovery following transportation (Ross *et al.*, 2009; Zahl *et al.*, 2012) [7, 8]. Among the various anaesthetic compounds used in aquaculture, eugenol, a major bioactive component of clove oil, has attracted considerable research interest because of its rapid sedative properties, relatively broad safety margin, biodegradability, economic affordability, and compara

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tively low environmental impact (Keene *et al.*, 1998; Woody *et al.*, 2002; Bagheri *et al.*, 2025) [10, 11, 9]. Furthermore, the antioxidant, anti-inflammatory, and antimicrobial activities associated with eugenol may provide additional benefits in attenuating some of the physiological disturbances induced by transportation stress (Pramod *et al.*, 2010) [12].

Despite these beneficial characteristics, the direct application of free or non-encapsulated eugenol for prolonged fish transportation presents several practical limitations. Eugenol possesses low water solubility and a strongly hydrophobic character, which often necessitates the incorporation of appropriate solvents or dispersing agents to achieve adequate distribution in the aquatic medium. Once introduced into water, eugenol can rapidly interact with and partition into lipid-rich biological membranes, particularly the gill tissues, potentially producing differences in uptake and resulting in fluctuations in anaesthetic effectiveness. Exposure to excessively high concentrations or prolonged contact may additionally result in respiratory suppression, prolonged anaesthetic recovery, alterations in gill tissues, and increased mortality in sensitive fish species (Ross *et al.*, 2009; Zahl *et al.*, 2012; Bagheri *et al.*, 2025) [7, 8, 9]. These limitations indicate the necessity of developing an alternative delivery approach capable of regulating eugenol availability and maintaining a relatively stable sedative concentration throughout extended transportation.

Encapsulation using controlled-release delivery systems provides a promising means of addressing several of the limitations associated with direct eugenol application. Such systems can regulate the release of the active compound and potentially provide a more sustained supply of eugenol throughout the transportation period. Sodium alginate is particularly suitable as an encapsulating polymer because it is biodegradable, biocompatible, relatively non-toxic, and capable of producing hydrogel structures through ionic cross-linking with calcium ions (Lee *et al.*, 2012) [13]. The concentration of calcium chloride (CaCl₂) used during bead formation plays an important role in determining the degree of polymer cross-linking, structural characteristics, porosity, and subsequent release behaviour of encapsulated substances. Consequently, optimization of CaCl₂ concentration is critical for obtaining alginate beads with appropriate structural stability and controlled release characteristics. Although considerable research has examined eugenol as an immersion anaesthetic, comparatively limited information is available regarding the performance of free eugenol in comparison with alginate-encapsulated eugenol during prolonged live fish transportation, particularly under simulated open transportation conditions.

The rosy barb (*Pethia conchonius*) was selected as the experimental fish because of its relatively robust nature and suitability for controlled laboratory investigations of physiological responses associated with transportation stress. Accordingly, the present investigation was designed to compare the effects of conventional free eugenol and alginate-encapsulated eugenol beads formulated using different concentrations of calcium chloride (CaCl₂) during a 24-h simulated open transportation experiment. The study was intended to assess whether encapsulation could provide a more controlled and sustained delivery of eugenol and thereby offer a suitable alternative approach for reducing the physiological challenges associated with prolonged live fish transportation.

2. Materials and methods

2.1 Experimental fish and acclimation

Healthy rosy barb (*Pethia conchonius*) (average body weight: 0.25 ± 0.03 g; total length: 2.8 ± 0.2 cm) were procured from a commercial ornamental fish hatchery and acclimated for 14 days in glass aquaria supplied with continuously aerated dechlorinated freshwater. Water temperature (26 ± 1 °C), dissolved oxygen (>6.0 mg L⁻¹), and pH (7.2 ± 0.2) were maintained throughout the acclimation period. Fish were fed a commercial ornamental fish diet (35% crude protein) twice daily at approximately 3% body weight, and feeding was discontin-

ued 24 h before transportation to minimize metabolic waste production during the experiment (Wedemeyer, 1996; Harmon, 2009) [2, 3].

2.2 Experimental design and treatments

The experiment was conducted using a completely randomized design (CRD) to evaluate the efficacy of free and alginate-encapsulated eugenol formulations during a 24-h simulated open transportation trial using rosy barb (*Pethia conchonius*) as the experimental model. The transportation trial was performed in glass aquaria (9 × 6.6 × 6 inch) containing 4 L of dechlorinated freshwater without supplementary aeration. Ten healthy fish were randomly stocked into each aquarium, and each treatment was maintained in triplicate.

Five experimental treatments were established: T₁, without any anaesthetic T₂, free (non-encapsulated) eugenol added directly to the aquarium water; T₃, alginate-encapsulated eugenol beads prepared using 1% CaCl₂; T₄, alginate-encapsulated eugenol beads prepared using 2% CaCl₂; and T₅, alginate-encapsulated eugenol beads prepared using 5% CaCl₂.

The recommended sedative dose of eugenol for fish transportation is 50 µL L⁻¹ (de Oliveira *et al.*, 2019) [14]. Accordingly, 200 µL of free eugenol was added directly to each aquarium containing 4 L of water in the reference treatment (T₂). For the encapsulated treatments, the number of alginate beads required to deliver an equivalent amount of eugenol was calculated based on the entrapment efficiency of each formulation, following the method described by Abdalla *et al.* (2015) [15]. Thus, all treatments received an identical quantity of eugenol, with the mode of delivery (free versus alginate-encapsulated) being the only experimental variable.

Transportation stress was simulated by intermittent manual shaking of each aquarium at 15-min intervals throughout the 24-h experimental period to mimic the vibration and water movement encountered during open road transportation. No water exchange or supplementary aeration was provided during the experiment, allowing progressive changes in water quality under simulated transportation conditions. Similar laboratory-based transportation models have been employed for evaluating transportation-induced stress and the efficacy of sedative agents in fish (Berka, 1986; Wedemeyer, 1996; Harmon, 2009) [1, 2, 3].

2.3 Water quality analysis

Water samples were collected at 0, 12, and 24 h to determine water quality variables, including water temperature, dissolved oxygen (DO), pH, free carbon dioxide (CO₂), and total ammonia nitrogen (TAN), following the APHA (2017) [16] standard methods for the examination of water and wastewater. These parameters were selected because they are recognized indicators of water quality deterioration during live fish transportation (Berka, 1986; Colt, 2006) [1, 6].

2.4 Statistical analysis

Data are presented as mean ± standard error (SE) of three independent replicates. Statistical analyses were performed using SPSS (Version XX; IBM Corp., Armonk, NY, USA). Treatment means were compared using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1 Water temperature

Water temperature in all treatments started in a narrow range of 22.3–23.0 °C and rose progressively over the 24-hour transport period, reaching 25.0–26.0 °C at the final sampling (Table 1). The magnitude of increase was similar across all five treatments, and no treatment-related pattern was evident, indicating that none of the treatment used measurably influenced heat buildup within unit; temperature change over time was driven primarily by ambient conditions and fish metabolic heat rather than by the water treatment themselves

3.2 Dissolved oxygen

Dissolved oxygen declined in every treatment over the course of transport, from an initial range of 8.1-8.6 mg/L to markedly lower values at 24 h (Table 2). The rate of decline differed significantly among treatments ($p < 0.05$) from the 12-hour sampling onward. The

control (T_1) showed the steepest decline, falling to 1.56 ± 0.40 mg/L by 24 h, followed by T_2 (2.60 ± 0.28 mg/L) and T_5 (3.16 ± 0.28 mg/L). T_3 and T_4 preserved substantially higher DO levels, with T_4 retaining the highest final DO of 5.33 ± 0.28 mg/L, roughly 3.4 times that of the control.

Table 1. Changes in water temperature (°C) during transportation of rosy barb under five treatments.

Parameter	Group	Initial	12 Hours	24 Hours (Final)
Temperature (°C)	T_1	22.6 ± 0.57	24.3 ± 0.57	26.0 ± 1.00
	T_2	22.3 ± 0.57	24.0 ± 1.00	25.33 ± 0.57
	T_3	23 ± 1.00	24.3 ± 1.15	25.66 ± 1.15
	T_4	23 ± 1.00	24.3 ± 0.57	25.33 ± 0.57
	T_5	22.3 ± 0.57	24.6 ± 1.52	25.00 ± 1.00

Table 2. Changes in dissolved oxygen (mg/L) during transportation of rosy barb under five treatments.

Parameter	Group	Initial	12 Hours	24 Hours (Final)
Dissolved Oxygen (mg/L)	T_1	8.1 ± 0.76	3.30 ± 0.44^{ab}	1.56 ± 0.40^a
	T_2	8.3 ± 0.28	4.00 ± 0.86^{ab}	2.6 ± 0.28^b
	T_3	8.3 ± 0.50	5.00 ± 0.50^{cd}	4.16 ± 0.28^c
	T_4	8.5 ± 0.28	5.83 ± 0.28^d	5.33 ± 0.28^d
	T_5	8.6 ± 0.28	4.33 ± 0.30^{bc}	3.16 ± 0.28^b

NB: Mean values within same column with different superscripts differ significantly ($p < 0.05$); Data are expressed as mean $\pm$ SE ($n = 3$)

3.3 Free carbon dioxide

Free carbon dioxide increased sharply in all treatments, from a near-uniform initial value of about 2.5 mg/L to final concentrations between 11.33 and 15.76 mg/L (Table 3). The control (T_1) again showed

the greatest CO_2 accumulation (15.76 ± 0.25 mg/L at 24 h), while T_4 limited the rise most effectively (11.33 ± 0.28 mg/L), with T_3 and T_5 intermediate and statistically similar to one another.

Table 3. Changes in free carbon dioxide (mg/L) during transportation of rosy barb under five treatments.

Parameter	Group	Initial	12 Hours	24 Hours (Final)
Carbon Dioxide (mg/L)	T_1	2.5 ± 0.11	11.73 ± 0.21^d	15.76 ± 0.25^C
	T_2	2.5 ± 0.05	11.46 ± 0.05^{cd}	15.40 ± 0.40^c
	T_3	2.5 ± 0.11	10.16 ± 0.28^b	13.33 ± 0.28^b
	T_4	2.5 ± 0.05	9.30 ± 0.32^a	11.33 ± 0.28^a
	T_5	2.6 ± 0.15	11.16 ± 0.28^c	13.83 ± 0.28^b

NB: Mean values within same column with different superscripts differ significantly ($p < 0.05$); Data are expressed as mean $\pm$ SE ($n = 3$)

3.4 pH

pH fell progressively in all treatments, from an initial 6.56-6.70 to final values of 4.06-5.70 (Table 4), mirroring the pattern of CO_2 accumulation described above. The steepest decline occurred in the con-

trol (T_1 , final pH 4.06 ± 0.05) and T_2 (final pH 4.10 ± 0.10), whereas T_4 maintained the highest final pH (5.70 ± 0.17), followed by T_5 (5.16 ± 0.15) and T_3 (4.56 ± 0.20).

Table 4. Changes in pH during transportation of rosy barb under five treatments.

Parameter	Group	Initial	12 Hours	24 Hours (Final)
pH (level)	T_1	6.56 ± 0.05	5.13 ± 0.05	4.06 ± 0.05^a
	T_2	6.6 ± 0.10	5.26 ± 0.05^a	4.10 ± 0.10^a
	T_3	6.6 ± 0.17	5.70 ± 0.17^b	4.56 ± 0.20^b
	T_4	6.7 ± 0.17	6.16 ± 0.15^c	5.70 ± 0.17^d
	T_5	6.6 ± 0.10	5.50 ± 0.30^{ab}	5.16 ± 0.15^c

NB: Mean values within same column with different superscripts differ significantly ($p < 0.05$); Data are expressed as mean $\pm$ SE ($n = 3$)

3.5 Total ammonia

Total ammonia was negligible and statistically similar across treatments at the start of the experiment (0.0013 - 0.0016 mg/L) but rose substantially by 24 h in all treatments (Table 5). The control (T_1) ac-

cumulated the most ammonia (0.108 ± 0.003 mg/L), followed by T_2 (0.092 ± 0.003 mg/L). T_3 and T_5 limited ammonia accumulation to 0.055 ± 0.003 and 0.049 ± 0.001 mg/L respectively, while T_4 again performed best, restricting final ammonia to 0.034 ± 0.003 mg/L — less than one-third of the control value.

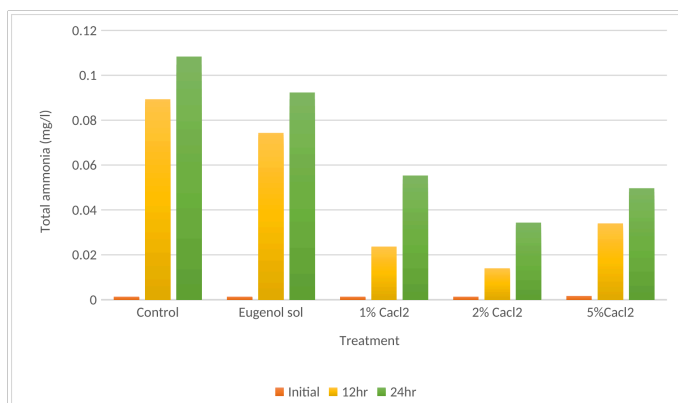


Figure 1. Changes in total ammonia (mg/L) during transportation of rosy barb under five treatments.

Taken together, the results show a consistent ranking of treatment performance across DO, CO₂, pH and ammonia, from best to worst maintenance of water quality: $T_4 > T_3 \approx T_5 > T_2 > T_1$ (control). Only water temperature was unaffected by treatment.

4. Discussion

The progressive rise in water temperature observed across all treatments is consistent with the general behaviour of sealed transport containers, in which heat generated by fish metabolism and the ambient environment accumulates in the absence of active cooling or water exchange (Luz *et al.*, 2024) [17]. Because none of the treatments tested targets thermal exchange, the similarity of the temperature trend across treatments was expected and indicates that any water-quality benefits attributable to the additives operated independently of temperature.

The steady decline in dissolved oxygen recorded in every treatment reflects the combined effects of fish respiration and, at least in the untreated control, unmitigated microbial oxygen demand within the closed system (Bulbul *et al.*, 2022; Ip and Chew, 2010) [18, 19]. As DO concentration falls, fish must compensate through increased ventilation, and beyond critical thresholds this can trigger anaerobic metabolism, elevated cortisol and broader stress responses (Barton, 2002; Wedemeyer, 1996).

The comparatively higher DO retained in T_4 is best, which suppresses locomotor activity and, consequently, metabolic rate and oxygen consumption - a mechanism widely reported for anaesthetic and sedative use during fish transport (Luz *et al.*, 2024; Cole, 1999) [17, 20]. The intermediate performance of T_3 limit the oxygen demand associated with nitrogenous waste, rather than a direct effect on fish respiration.

The marked accumulation of free CO₂ and the corresponding decline in pH observed across treatments are mechanistically linked: as dissolved CO₂ rises, carbonic acid formation lowers the pH of poorly buffered transport water, a pattern repeatedly documented in closed-system fish transport (Moran *et al.*, 2008; Sampaio *et al.*, 2016; Harmon, 2009) [21, 22, 3]. Elevated CO₂ (hypercapnia) can itself impair oxygen uptake at the gill and induce respiratory acidosis, compounding the stress associated with low DO (Moran *et al.*, 2008) [21]. The superior performance of T_4 in limiting both CO₂ buildup and pH decline is again consistent with sedation-induced suppression of aerobic respiration, since CO₂ production, like oxygen consumption, scales with metabolic rate. T_3 and T_5 produced intermediate benefits, plausibly through their respective effects on ammonia adsorption and oxidation of organic waste, which can indirectly reduce the microbial and chemical processes that generate additional CO₂ in transport water.

Total ammonia accumulation followed a similar treatment ranking. Ammonia is excreted continuously across the gill epithelium as the principal nitrogenous waste product of protein catabolism in teleost fish, and its accumulation in closed transport systems is well documented as a major cause of transport-related mortality (Randall *et al.*,

2002; Eddy, 2005; Ip *et al.*, 2010) [24, 23, 19]. Critically, the toxicity of total ammonia is governed by the proportion present as un-ionised ammonia (NH₃), which increases sharply with rising pH (Randall *et al.*, 2002) [24]. The concurrent decline in pH observed in the present study would therefore have partially offset the toxicological impact of rising total ammonia by shifting the ammonia/ammonium equilibrium toward the less toxic ionised form; nevertheless, the substantially lower total ammonia recorded in T_4 and T_3 indicates a genuine reduction in ammonia loading rather than merely a pH-driven artefact.

Maintenance of transport water quality is a major determinant of successful live fish transportation. In the present study, dissolved oxygen declined whereas carbon dioxide and total ammonia nitrogen increased progressively in all treatments, reflecting continuous respiration and nitrogenous waste excretion under confined conditions. However, fish treated with the 2% CaCl₂ formulation maintained significantly higher dissolved oxygen together with lower carbon dioxide and ammonia concentrations than fish receiving free eugenol. These findings indicate that controlled release of eugenol effectively reduced metabolic activity throughout the transportation period, thereby lowering oxygen consumption and reducing the accumulation of respiratory and metabolic wastes. Similar improvements in transport water quality following effective sedation have been reported during live fish transportation, where reduced locomotor activity lowers oxygen demand and ammonia production while slowing deterioration of the transport environment (Berka, 1986; Wedemeyer, 1996; Harmon, 2009) [1, 2, 3]. The comparatively stable pH observed in the optimized formulation further supports reduced carbon dioxide accumulation and improved buffering conditions during transportation.

5. Conclusion

The present study showed that entrapping eugenol in alginate beads improved its utility for long-duration live fish transport by enabling gradual, regulated release of the sedative. Of the formulations examined, beads cross-linked with 2% CaCl₂ offered the best balance of water quality parameter. This study evaluated the effect of eugenol-loaded alginate beads, cross-linked at three CaCl₂ concentrations, on water quality during a 24-h simulated live transport trial. Initial values of all measured parameters were statistically comparable across treatments, and water temperature rose uniformly by approximately 3 °C irrespective of treatment, confirming that the observed differences arose from treatment rather than from thermal variation. All other parameters deteriorated progressively with time, but to markedly different degrees. Beads cross-linked with 2% CaCl₂ consistently maintained the most favourable transport medium, sustaining dissolved oxygen at 5.33 mg/L at 24 h against 1.56 mg/L in the untreated control, while limiting free carbon dioxide to 11.33 mg/L, restricting the pH decline to 5.70, and holding total ammonia to 0.034 mg/L, roughly one-third of the control value. The response to cross-linking density was non-monotonic: performance improved from 1% to 2% but declined again at 5%, indicating an optimum in the region of 2%. Free eugenol gave only a marginal improvement over the control and was outperformed by all three encapsulated formulations. It is therefore concluded that encapsulation of eugenol in alginate beads cross-linked with 2% CaCl₂ offers an effective, simple and practical means of conserving water quality during closed-system transport of up to 24 h, and merits validation at commercial scale and across additional aquaculture species.

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How to cite this article: Debnath A, Sau SK, Biswas P, Dhara H, Deb S, Das SK, et al. Effect of water quality parameters in the transportation of Rosy Barb (*Pethia conchonius*) with eugenol encapsulated beads. *International Journal of Advanced Biochemistry Research*. 2026;10(9):871-875. doi: <https://www.doi.org/10.33545/26174693.2026.v10.i9k.10204>

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