

E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2023.36.1.001

# Optimisation of Safe Loading Density for Live Transportation of Rainbow Trout, *Oncorhynchus mykiss* (Walbaum, 1792), Yearlings in Plastic Bags

MANCHI RAJESH*, BIJU SAM KAMALAM, MANEESH KUMAR DUBEY, PARVAIZ AHMAD GANIE, KISHOR KUNAL
ICAR-Directorate of Coldwater Fisheries Research, Bhimtal 263136, Uttarakhand, India

*E-mail: Rajesh.M@icar.gov.in | Received: 04/10/2022; Accepted: 14/01/2023

## Abstract

A field study evaluated the safe loading density for live transportation of rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), yearlings in plastic bags of super-oxygenated water. The experimental fish (197.5 ± 40 g) were starved for 72 h and mildly sedated with clove oil (40 μL.L$^{-1}$ for 2–3 min) before packing. Three loading densities of live rainbow trout (120, 170, 230 g.L$^{-1}$) were packed in plastic bags containing 6 L of stream water and 10–12 L of medical-grade oxygen gas. Six replicate bags per loading density were then subjected to 10 h of transportation by road at ~10 °C. On arrival at the destination, important water quality indicators and fish survival were recorded from each plastic bag. With increasing fish loading density, there was a corresponding increase in the concentrations of carbon dioxide ($CO_2$), total ammonia nitrogen (TAN), free ammonia ($NH_3$), total dissolved solids (TDS) concentrations and specific conductivity in the transport water, and a decrease in dissolved oxygen (DO, $R^2$ = 0.91-0.99). These differences in vital water quality indicators were significantly more in the 230 g.L$^{-1}$ group. However, there was no mortality even at the highest loading density, as the critical water quality parameters did not cross the acute lethal thresholds for rainbow trout. Based on the findings, it is recommended that a loading density of 230 g.L$^{-1}$ is safe for live transportation of rainbow trout yearlings in plastic bags over a transport duration of 10 h at ~10 °C, following adequate starvation and mild sedation.

**Keywords:** live fish transportation, loading density, water quality, total ammonia nitrogen, rainbow trout

## Introduction

Transportation of live fish is an essential activity of an aquaculture enterprise. While small young juveniles are often transported for stocking purposes, yearlings and sub-adults are occasionally transported for brooder development programmes, research purposes and live fish sale in the market. Transportation involves very stressful conditions, where fish are exposed to deteriorating water quality environment, crowding, handling and acute physical turbulences. This invariably affects the key physiological processes of respiration, excretion and osmoregulation, resulting in disruption of homeostasis (Barton, 2000; King, 2009; Harmon, 2009; Tacchi et al., 2015; Sampaio and Freire, 2016). Fish mortality occurs when transportation conditions cross the acute tolerance limits, resulting in economic losses. Therefore, it is essential to ascertain safe or optimum loading densities for different sizes of each fish species for temperature, transport duration, transportation system (Gomes et al., 2003; Pavlidis et al., 2003; Hasan and Bart, 2007; Stieglitz et al., 2012, Hong et al., 2019; Vanderzwalmen et al., 2021) and feed and water additives (Tacchi et al., 2015; Sampaio and Freire, 2016; Vanderzwalmen et al., 2019) to ensure the success of live fish transportation.

Different transportation systems are utilised for shipping live fish, such as insulated tanks in trucks fitted with an oxygen cylinder and a chilling facility (Falconer, 1964); sealed containers such as polyethene bottles or plastic bags inflated with pure oxygen (Gebhards, 1965; Berka, 1986); and well boats or towing of culture cages in coastal areas (Carmichael and Tomasso, 1988; King, 2009). The choice of transportation system depends on the system's availability, duration of the transport, road connectivity, geographical location and the number of fish to be transported.

For small-scale live fish transportation, using plastic bags filled with water and oxygen is economical and is common practice for prolonged transportation (>24 h). It does not require a dedicated transport vehicle (fish transportation van/truck) and can be shipped through public transport (Berka, 1986; Lim et al., 2003). Further, it is a convenient method for transporting fish to geographically rugged terrains and remote farm locations without road connectivity, as commonly observed in the Indian Himalayan region.

For deciding safe loading density, theoretically calculated tables are available to transport rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), up to 20 g in size, for different transport duration (5-50 h) and temperature conditions (Berka, 1986). Studies focused on the physiological impact of transportation have evaluated loading densities of 60-420 g.L$^{-1}$ rainbow trout for transport duration of ≤8 h in different live transportation systems (Gebhards, 1965; Barton and Peter, 1982; Piper et al., 1982; Floss, 1988; Möck and Peters, 1990; Barton, 2000; Leggatt et al., 2006). A survey in the United States of America has also reported the use of a range of loading densities from 20-420 g.L$^{-1}$ for transportation of rainbow trout (<1-16 h) under commercial conditions (Carmichael and Tomasso, 1988).

A previous study evaluated the transport of rainbow trout in plastic bags or closed systems in developing countries with optimisation of different factors such as the duration of starvation, the concentration of clove oil for mild sedation, the addition of salt and safe loading density for transporting rainbow trout fingerlings of 4 g for a long hauling duration of 40 h at 13 °C (Kamalam et al., 2017).

Considering the lack of information on the optimal conditions for transporting bigger individuals of rainbow trout in plastic bags, the present study was carried out using yearlings (~200 g) to evaluate three loading densities for a transport duration of 10 h. The results of the study were based on changes in water quality and fish survival.

# Materials and Methods

## *Ethical approval*

The experiment was conducted with the consent of the ethical committee of the ICAR-Directorate of Coldwater Fisheries Research, Bhimtal (DCFR/IACUC /12/06/2020/4). All protocols involving the use of fish were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines, Ministry of Environment and Forests, Government of India.

## *Experimental fish and source water quality*

The present study was carried out in December. Fish were transported from the experimental fish farm of ICAR-Directorate of Coldwater Fisheries Research at Champawat (latitude 29°17'52.152"N; longitude 80°6'42.804"E; altitude 1400 msl) to recirculating aquaculture system facility at the headquarters in Bhimtal, Uttarakhand, India (latitude 29°21'37.283"N; longitude 79°33'10.316"E; altitude 1350 msl). Prior to shipment, the experimental rainbow trout were reared in a flow-through raceway (25 m$^3$) provided with continuous water supply from a nearby spring-fed rivulet and were fed a fish meal-based pelleted diet (4 mm) containing 45 % crude protein and 16 % crude lipid.

## *Packing and transportation*

Live rainbow trout were packed as per the standardised protocol from the previous study (Kamalam et al., 2017). On the day of packing, 72 h starved fish were hauled from the raceways using a dragnet, and individual fish of 197.5 ± 40 g size were randomly taken out using a hand net. The fish were weighed, counted and mildly anaesthetised using clove oil (40 µL.L$^{-1}$, ≥85 % eugenol, CDH Chemicals, India) for about 2-3 min (until they lost equilibrium). After partial recovery in running freshwater, they were quickly transferred into the respective plastic bags (3–7 fish per bag) to obtain different loading densities (120, 170 and 230 g.L$^{-1}$). Twenty-five litre plastic bags with a dimension of 0.78 × 0.48 m (tightly tied at the bottom using jute rope before packing) were used in double for fish packing.

Bags were initially filled with 6 L of water and checked for leakage. Immediately after the transfer of fish into respective bags, nearly 10–12 L (~1:2 ratio of water to oxygen) oxygen from cylinder was introduced, and the bag was tightly sealed using jute rope (Fig. 1). Eighty-six fish were packed in 18 bags, i.e., six bags for each loading density (n = 6). For control measurement, another plastic bag was packed without fish. The packed plastic bags were then placed in individual corrugated polystyrene boxes (0.25 × 0.25 × 0.4 m) to prevent any damage to the bags during transportation.

The experimental live fish consignment was transported in a van (Toyota Innova, Japan), covering a total road distance of 160 km through hilly terrain (>1300 msl; Fig. 2). As the outside weather condition during the transportation was cold, there was no requirement to switch on the vehicle's air-conditioning for temperature control. The complete shipment process (i.e., the start of the live fish packing, transportation and until the final release into the tank) took around 10 h (11:00 a.m. – 09:00 p.m.).

## *Survival and water quality analysis*

At the destination, immediately after opening each

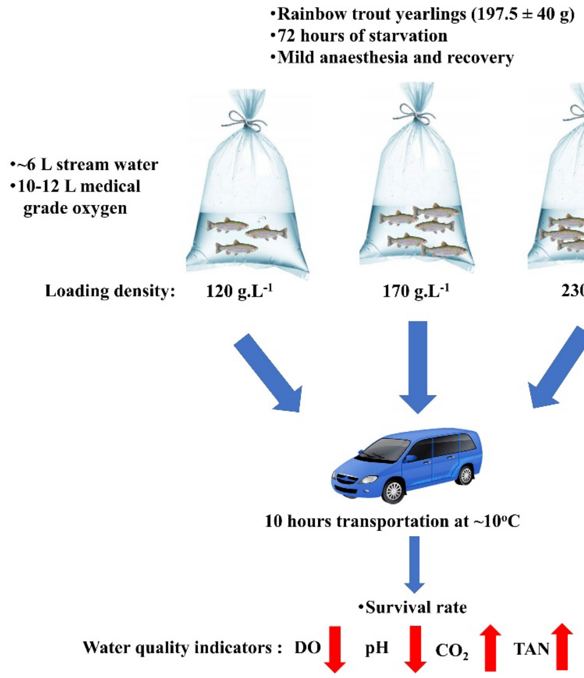


Fig. 1. Schematic representation of the experimental design and methodology of transporting rainbow trout, *Oncorhynchus mykiss.*

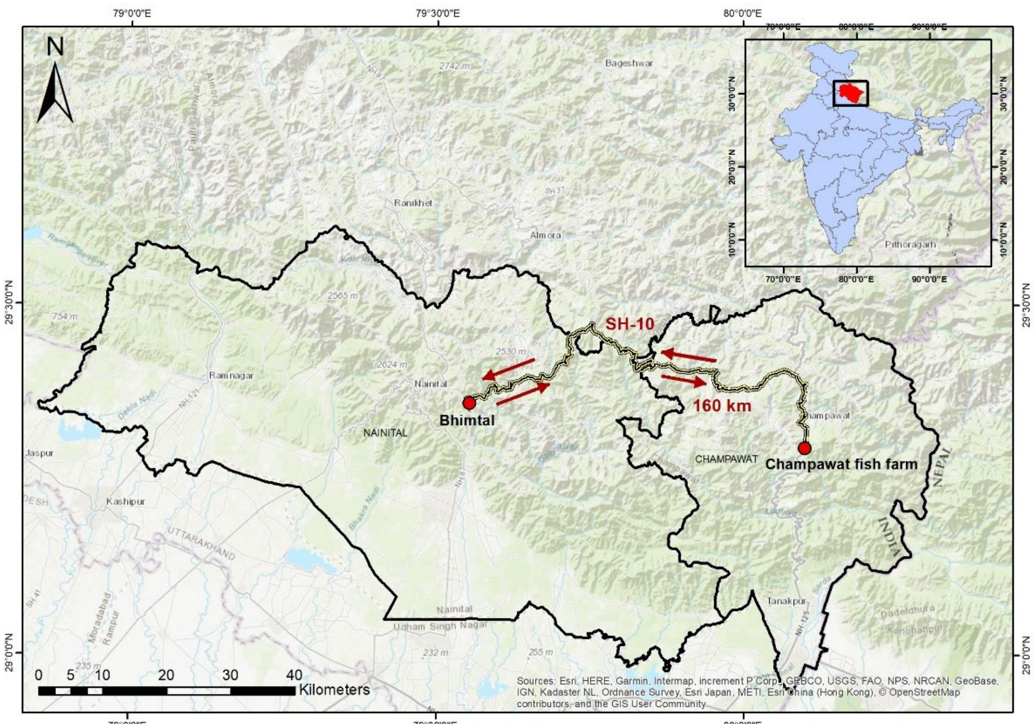


Fig. 2. Geographical location and route of transportation of rainbow trout, *Oncorhynchus mykiss.*

plastic bag, water quality parameters, namely, temperature, dissolved oxygen, TDS, specific conductivity, pH and salinity, were measured using a portable multi-parameter digital water quality meter (ProDSS, YSI, USA). Carbon dioxide and alkalinity were measured using commercial titration kits (HiMedia, India). Turbidity was analysed using a portable turbidimeter (2100Q Hach, USA). To estimate total ammonia nitrogen (TAN), 15 mL water sample was collected from each bag and stored in a -20 °C freezer. TAN was quantified using the salicylate-hypochlorite method (Bower and Holm-Hansen, 1980), standardised to a microplate-based procedure. The free ammonia ($NH_3$) was determined from TAN values using temperature and pH equilibrium tables. The bags were checked for any fish loss or mortality, and the water volume in each bag was measured precisely before releasing the fish into the receiving tanks.

## *Statistical analysis*

Statistical analysis of the data set was performed using one-way ANOVA in Graphpad prism (version 6.01 for Windows, USA), followed by post hoc multiple comparisons (Tukey's test). Probability values of <0.05 were considered statistically significant, and the results were expressed as mean ± standard deviation. Linear regression analysis was also performed between fish loading density and each water quality parameter. The correlation coefficients and regression equations are provided along with the data (Table 1).

# Results

On the day of packing, water quality parameters of the source water used for rearing and packing fish were as follows, temperature 8.1 °C, dissolved oxygen 9.1 mg.L$^{-1}$, alkalinity 20 mg.L$^{-1}$ $CaCO_3$, pH 7.2, carbon dioxide 12 mg.L$^{-1}$, total ammonia nitrogen (TAN) 0.06 mg.L$^{-1}$, total dissolved solids (TDS) 29 mg.L$^{-1}$, conductivity 31.4 μS.cm$^{-1}$, turbidity 0.79 NTU and salinity 0.02 g.L$^{-1}$. On arrival at the destination, important water quality indicators and fish survival were recorded from each plastic bag. As compared to the control (bag without fish), water quality in all the fish carrying bags showed a decrease in dissolved oxygen and pH, and simultaneous increase in carbon dioxide, total ammonia nitrogen, free ammonia, turbidity, total dissolved solids (TDS) and conductivity (Table 1). With increasing fish loading density, there was a corresponding increase in $CO_2$, TAN, $NH_3$, TDS concentrations and specific conductivity in the transport water; and a decrease in DO ($R^2$ = 0.91-0.99; Table 1). These differences in vital water quality indicators were significantly ($P < 0.05$) more in the 230 g.L$^{-1}$ group. No mortality was observed even at the highest loading density, as the critical water quality parameters did not cross the acute lethal thresholds for rainbow trout.

# Discussion

For optimising the transportation of rainbow trout yearlings in plastic bags with super-oxygenated water, in this study, we evaluated three loading densities of 120, 170 and 230 g.L$^{-1}$ for a transport duration of 10 h at ~10 °C (Table 1). At the end of the transportation procedure, irrespective of the loading density, there was a decrease in DO and pH with a corresponding rise in $CO_2$ level in the fish transported water, as compared to the bag without fish (DO, 37.7 mg.L$^{-1}$; oxygen saturation, 383 %; pH, 7.24; and $CO_2$ 14 mg.L$^{-1}$). Similarly, TAN, $NH_3$, turbidity, TDS and conductivity levels increased by many folds in the fish transported water, compared to the control (TAN, 0.06 mg.L$^{-1}$; turbidity, 0.7 NTU; TDS, 29 mg.L$^{-1}$; and conductivity, 31.4 μS.cm$^{-1}$). The above changes are commonly observed during live fish transportation, as oxygen is consumed during respiration. The drop in pH is mainly due to the build-up of $CO_2$, and the increased TAN level

Table 1. Rainbow trout, *Oncorhynchus mykiss*, survival and changes in water quality due to different fish loading densities after 10 hours of transportation.

| Parameter | Control | Fish loading density (g.L$^{-1}$) | | | *P*-value (ANOVA) | Linear regression | |
|---|---|---|---|---|---|---|---|
| | | 120 | 170 | 230 | | $R^2$ | *P*-value |
| Fish loading density (g.L$^{-1}$) | - | 121 ± 2$^a$ | 171 ± 1$^b$ | 229 ± 3.2$^c$ | < 0.0001 | - | - |
| Temperature (°C) | 8.9 | 9.3 ± 0.4$^a$ | 9.9 ± 0.6$^b$ | 10 ± 0.2$^b$ | 0.02 | 0.85 | 0.26 |
| Oxygen saturation (%) | 325 | 202 ± 49.3$^b$ | 160 ± 17$^a$ | 144 ± 9.9$^a$ | 0.01 | 0.91 | 0.19 |
| Dissolved oxygen (mg.L$^{-1}$) | 37.68 | 19.7 ± 0.4$^b$ | 15.4 ± 1.7$^a$ | 13.8 ± 0.95$^a$ | 0.011 | 0.92 | 0.19 |
| Carbon dioxide (mg.L$^{-1}$) | 12 | 24.9 ± 2.3$^a$ | 30.4 ± 5.2$^b$ | 31 ± 3.5$^b$ | 0.01 | 0.88 | 0.22 |
| pH | 7.24 | 6.14 ± 0.12 | 6.12 ± 0.09 | 6.1 ± 0.13 | 0.76 | 0.99 | 0.02 |
| Total ammonia nitrogen (mg.L$^{-1}$) | 0.06 | 7.1 ± 1.5$^a$ | 8.7 ± 0.5$^{ab}$ | 9.7 ± 0.1$^b$ | 0.002 | 0.98 | 0.1 |
| Free $NH_3$ (μg.L$^{-1}$) | 0.17 | 1.5 ± 0.4$^a$ | 2.1 ± 0.3$^{ab}$ | 2.3 ± 0.1$^b$ | 0.009 | 0.91 | 0.2 |
| Turbidity (NTU) | 0.79 | 5.7 ± 2.9 | 6.4 ± 1.1 | 6.4 ± 1.2 | 0.78 | 0.7 | 0.4 |
| Specific conductivity (μS.cm$^{-1}$) | 45.4 | 154.7 ± 24$^a$ | 181.4 ± 12.5$^b$ | 205.2 ± 10.94$^b$ | 0.02 | 0.99 | 0.05 |
| Total dissolved solids (mg.L$^{-1}$) | 29 | 100.5 ± 15.6$^a$ | 118 ± 7.9$^{ab}$ | 133.5 ± 7.2$^b$ | 0.002 | 0.99 | 0.05 |
| Salinity (g.L$^{-1}$) | 0.02 | 0.076 ± 0.01$^a$ | 0.086 ± 0.01$^{ab}$ | 0.095 ± 0.006$^b$ | 0.01 | 0.99 | 0.05 |
| Survival (%) | - | 100 | 100 | 100 | - | - | - |

Data are presented as mean ± standard deviation (n = 6 bags / loading density). Different superscript letters in the same row indicate statistically significant differences in water quality parameters between the different loading densities at $P < 0.05$ (one-way ANOVA followed by Tukey's multiple comparison test). Linear regression equations obtained for the major water quality indices are as follows: $CO_2$ (mg.L$^{-1}$), Y = 0.05536 × X + 19.15; dissolved oxygen saturation (%) at Y = - 0.5280 × X + 260.6; dissolved oxygen (mg.L$^{-1}$), Y = - 0.05365 × X + 25.61; TAN (mg.L$^{-1}$), Y = 0.02415 × X + 4.274; $NH_3$ (μg.L$^{-1}$), Y = 0.007587 × X + 0.6591; pH, Y = - 0.0003986 × X + 6.192, *Control refers to plastic bags without fish.

from 0.06 mg.L$^{-1}$ to 7-9 mg.L$^{-1}$ (Table 1) is due to the release of ammonia, a by-product of nitrogen metabolism in fishes (Colt and Armstrong 1981; Harmon, 2009; Kamalam et al., 2017). As fishes were starved for 72 h prior to packing and transportation, intact faecal matter was not noticed. Still, there was a rise in water turbidity, which could be due to the sloughing of mucus (Harmon, 2009; Tacchi et al., 2015). The increase in TDS, salinity and specific conductivity can be due to increased concentration of solutes such as ammonia and catecholamine-induced passive solute loss from fish into the water and increased carbon dioxide concentration in water (King, 2009; Harmon, 2009).

With increasing fish loading density from 120 to 230 g.L$^{-1}$, there was a significant decrease in DO and an increase in $CO_2$, TAN, $NH_3$, TDS and conductivity levels in the transport water (Table 1, $R^2$ = 0.91-0.99). In general, the quality of transport water was found to deteriorate more with increasing loading density. However, there was no immediate mortality or weakened fish in any tested loading densities. This could be attributed to the fact that the vital water quality indicators were not close to the lethal thresholds, i.e., DO < 3 mg.L$^{-1}$, $CO_2$ 60-70 mg.L$^{-1}$ and free $NH_3$ >10 μg.L$^{-1}$ (Piper et al., 1982; Berka, 1986; Wedemeyer, 1996; Molony, 2001), even in the highest loading density (230 g.L$^{-1}$), under the present experimental transport conditions. It should be noted that the complex interactions between the different water quality variables also determine fish survival. For instance, the toxicity of $CO_2$ is normally high at lower pH, and rainbow trout mortalities can occur at 20 mg.L$^{-1}$ $CO_2$ at 5.7 pH (Lloyd and Jordan, 1964). But, at the same time, high DO levels have been found to offset the toxicity of $CO_2$ (Alabaster et al., 1957; Piper et al., 1982). In the present study, even at the highest loading density, pH did not cross the threshold level of 6, and high DO levels (144 % sat) could be the reason for the fish survival despite elevated $CO_2$ in transported water (Berka, 1986).

For TAN, the permissible level for the culture of rainbow trout is ≤1 mg.L$^{-1}$(Timmons et al., 2018). Moreover, the toxicity of ammonia depends on pH and temperature, as they determine the ratio of gaseous or free ammonia ($NH_3$, toxic form, as the gill membrane is highly permeable to $NH_3$) and the cationic form ($NH_4^+$, not toxic, due to lower permeability) in water (Ip and Chew, 2010). In the current study, though TAN levels in the highest loading density reached 9.7 mg.L$^{-1}$ (Table 1), the free ammonia level ($NH_3$) was below the toxic threshold of >0.01 mg.L$^{-1}$ (10 ug.L$^{-1}$) due to the lower pH of transport water. As reported in previous investigations, the pre-transport procedures of fasting for 72 h and mild anaesthetisation in rainbow trout also would have significantly reduced ammonia excretion due to lowering of metabolic rate (Phillips and Brockway, 1954; Kamalam et al., 2017; Vanderzwalmen et al., 2019).

In a previous study in rainbow trout fingerlings, 26.7 g.L$^{-1}$ loading density was suitable for a long haul of 40 h at 13 °C (Kamalam et al., 2017). Whereas in this study, based on the survival of the rainbow trout yearlings, even the highest loading density of 230 g.L$^{-1}$ was viable for 10 h transport duration at ~10 °C. Interestingly, this density is 2.5 times higher than the theoretically calculated and recommended loading density of 90 g.L$^{-1}$ for transportation of juvenile salmonids in plastic bags for 10 h duration at 10 °C (Berka,1986). The pre-shipment procedures that were followed (72 h of starvation and mild anaesthesia) and the size of individual fish (200 vs 20 g) could be possible reasons for the substantial difference between this study and the previous theoretical calculation.

## Conclusion

This is the first practical study to evaluate the safe loading density for the transport of rainbow trout yearlings (~200 g) in plastic bags with super-oxygenated water. Based on fish survival and physico-chemical changes in the transport medium where water quality indices did not reach acute toxic levels, a high loading density of 230 g.L$^{-1}$ could be viably used for the transportation of rainbow trout, *Oncorhynchus mykiss* yearlings for 10 h duration at ~10 °C, after a 72 h starvation period and mild anaesthetisation before packing.

## Acknowledgements

The authors are grateful to Dr. Debajit Sarma and Director (In-charge), ICAR-Directorate of Coldwater Fisheries Research, for providing necessary facilities and support to successfully carry out this study. The funding support provided by the Indian Council of Agricultural Research for this study, under the institutional project AQ21 is gratefully acknowledged. We also thank Mr. Ravindra Posti for preparing route map and the supporting staffs of the experimental fish farm, ICAR-DCFR, Champawat, and Mrs. Shriya R for their assistance in fish packing and sampling.

Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Manchi Rajesh: Conceptualisation, methodology, investigation, formal analysis, writing original draft. Biju Sam Kamalam: Conceptualisation, methodology, writing, review and editing. Maneesh Kumar Dubey: Investigation. Parvaiz Ahmad Ganie: Investigation and software. Kishor Kunal: Resources and investigation. All the authors read and approved the final manuscript.

## References

Alabaster, J.S., Herbert, D.W.M., Memens, J. 1957. The survival of rainbow trout (*Salmo gairdneri* Richardson) and perch (*Perca fluviatilis* L.) at various concentrations of dissolved oxygen and carbon dioxide.

Annals of Applied Biology 45:177–188. https://doi.org/10.1111/j.1744-7348.1957.tb00452.x

Barton, B.A. 2000. Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. North American Journal of Aquaculture 62:12–18. https://doi.org/10.1577/1548-8454(2000)062<0012:SFDITC>2.0.CO;2

Barton, B.A., Peter, R.E. 1982. Plasma cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* Richardson, to various transport conditions, anaesthesia, and cold shock. Journal of Fish Biology 20:39–51. https://doi.org/10.1111/j.1095-8649.1982.tb03893.x

Berka, R. 1986. The transport of live fish: a review. EIFAC Technical Paper, 48. Food and Agriculture Organization of the United Nations, Rome. 52 pp.

Bower, C.E., Holm-Hansen, T. 1980. A salicylate-hypochlorite method for determining ammonia in seawater. Canadian Journal of Fisheries and Aquatic Sciences 37:794–798. https://doi.org/10.1139/f80-106

Carmichael, G.J., Tomasso, J.R. 1988. Communications: Survey of fish transportation equipment and techniques. The Progressive Fish-Culturist 50:155–159. https://doi.org/10.1577/1548-8640(1988)050<0155:CSOFTE>2.3.CO;2

Colt, J.E., Armstrong, D.A. 1981. Nitrogen toxicity to crustaceans, fish, and molluscs. In: Proceedings of the bio-engineering symposium for fish culture (eds. Allen, L.J., Kinney, E.C.), pp. 34–47. American Fisheries Society, Bethesda, USA.

Falconer, D.D. 1964. Practical trout transport techniques. The Progressive Fish-Culturist 26:51–58. https://doi.org/10.1577/1548-8659(1964)26[51:PTTT]2.0.CO;2

Flos, R., Reig, L., Torres, P., Tort, L. 1988. Primary and secondary stress responses to grading and hauling in rainbow trout, *Salmo gairdneri*. Aquaculture 71:99–106. https://doi.org/10.1016/0044-8486(88)90277-3

Gebhards, S.V. 1965. Transport of juvenile trout in sealed containers. The Progressive Fish-Culturist 27:31–36. https://doi.org/10.1577/1548-8640(1965)27[31:TOJTIS]2.0.CO;2

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

Harmon, T.S. 2009. Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. Reviews in Aquaculture 1:58–66. https://doi.org/10.1111/j.1753-5131.2008.01003.x

Hasan, M., Bart, A.N. 2007. Effects of capture, loading density and transport stress on the mortality, physiological responses, bacterial density and growth of rohu *Labeo rohita* fingerlings. Fish Physiology and Biochemistry 33:241–248. https://doi.org/10.1007/s10695-007-9136-7

Hong, J., Chen, X., Liu, S., Fu, Z., Han, M., Wang, Y., Gu, Z., Ma, Z., 2019. Impact of fish density on water quality and physiological response of golden pompano (*Trachinotus ovatus*) flingerlings during transportation. Aquaculture 507:260–265. https://doi.org/10.1016/j.aquaculture.2019.04.040

Ip, A.Y., Chew, S.F. 2010. Ammonia production, excretion, toxicity, and defense in fish: a review. Frontiers in Physiology 1:134. https://doi.org/10.3389/fphys.2010.00134

Kamalam, B.S., Patiyal, R.S., Rajesh, M., Mir, J.I., Singh, A.K. 2017. Prolonged transport of rainbow trout fingerlings in plastic bags: optimisation of hauling conditions based on survival and water chemistry. Aquaculture 480:103–107. https://doi.org/10.1016/j.aquaculture.2017.08.012

King, H.R. 2009. Fish transport in the aquaculture sector: An overview of the road transport of Atlantic salmon in Tasmania. Journal of Veterinary Behavior 4:163–168. https://doi.org/10.1016/j.jveb.2008.09.034

Leggatt, R.A., Scheer, K.W., Afonso, L.O., Iwama, G.K. 2006. Triploid and diploid rainbow trout do not differ in their stress response to transportation. North American Journal of Aquaculture 68:1–8. https://doi.org/10.1577/A05-035.1

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

Lloyd, R., Jordan, D.H.M. 1964. Some factors effecting the resistance of rainbow trout (*Salmo giardnerii* Richardson) to acid waters. International Journal of Air and Water Pollution 8:393–403.

Möck, A., Peters, G. 1990. Lysozyme activity in rainbow trout, *Oncorhynchus mykiss* (Walbaum), stressed by handling, transport and water pollution. Journal of Fish Biology 37:873–885. https://doi.org/10.1111/j.1095-8649.1990.tb03591.x

Molony, B. 2001. Environmental requirements and tolerances of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) with special reference to Western Australia: A review. Fisheries Research Report No. 130. Department of Fisheries Perth, Western Australia. 28 pp.

Pavlidis, M., Angellotti, L., Papandroulakis, N., Divanach, P. 2003. Evaluation of transportation procedures on water quality and fry performance in red porgy (*Pagrus pagrus*) fry. Aquaculture 218:187–202. https://doi.org/10.1016/S0044-8486(02)00314-9

Phillips, A.M., Brockway, D.R. 1954. Effect of starvation, water temperature, and sodium amytal on the metabolic rate of brook trout. The Progressive Fish Culturist 16:65–68. https://doi.org/10.1577/1548-8659(1954)16[65:EOSWTA]2.0.CO;2

Piper, R.E., McElwain, I.B., Orme, L.E., McCraren, J.P., Fowler, L.G., Leonard, J.R. 1982. Fish hatchery management. U.S. Fish and Wildlife Service, Washington, DC. pp. 1-33.

Sampaio, F.D., Freire, C.A. 2016. An overview of stress physiology of fish transport: changes in water quality as a function of transport duration. Fish and Fisheries 17:1055–1072. https://doi.org/10.1111/faf.12158

Stieglitz, J.D., Benetti, D.D., Serafy, J.E. 2012. Optimizing transport of live juvenile cobia (*Rachycentron canadum*): effects of salinity and shipping biomass. Aquaculture 364:293–297. https://doi.org/10.1016/j.aquaculture.2012.08.038

Tacchi, L., Lowrey, L., Musharrafieh, R., Crossey, K., Larragoite, E.T., Salinas, I. 2015. Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 435:120–127. https://doi.org/10.1016/j.aquaculture.2014.09.027

Timmons, M., Guerdat, T., Vinci, B.J. 2018. Recirculating aquaculture, 4th Edition. Ithaca Publishing Company, LLC, Vero Beach, Florida, USA, pp. 27–90.

Vanderzwalmen, M., Eaton, L., Mullen, C., Henriquez, F., Carey, P., Snellgrove, D., Sloman, K.A. 2019. The use of feed and water additives for live fish transport. Reviews in Aquaculture 11:263–278. https://doi.org/10.1111/raq.12239

Vanderzwalmen, M., McNeill, J., Delieuvin, D., Senes, S., Sanchez-Lacalle, D., Mullen, C., McLellan, I., Carey, P., Snellgrove, D., Foggo, A., Alexander, M.E. 2021. Monitoring water quality changes and ornamental fish behaviour during commercial transport. Aquaculture 531:735860. https://doi.org/10.1016/j.aquaculture.2020.735860

Wedemeyer, G.A. 1996. Physiology of fish in intensive culture systems. Chapman and Hall, New York. 226 pp.