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ENHANCING THE SURVIVAL RATE IN LIVE FISH TRANSPORT BY UTILIZING NANOBUBBLE TECHNOLOGY

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ABSTRACT
Efficient transportation of live fish is crucial for aquaculture, necessitating methods to ensure that fish maximize survival rates. This study was carried out in March 2022 at the Rainbow Trout Fishery Research Center to evaluate various aeration systems and packing methods for transporting trout fingerlings for 12 hours, with a focus on nanobubble technology. Closed and open transport systems were tested using oxygenated, ozonized, and air nanobubbles, compared to conventional methods. In the closed system with 500-750 fish per bag, ozone nanobubbles achieved significantly ($P<0.05$) higher survival rates of 99.6±0.10 and 99.3±0.0 %, versus 64.5% with conventional oxygen packing. The ozone nanobubbles extended the duration until the dissolved oxygen reached 4 mg/L. For open transportation, oxygen nanobubbles with a droplet size of around 100-600 nm led to 99.0±0.10% survival, significantly ($P<0.05$) exceeding 76.7±5.20% with regular oxygen aeration. Crucially, nanobubble systems consistently maintained elevated dissolved oxygen (8-9 mg/L) and reduced ammonia concentrations (0.01-0.03 mg/L), indicating superior fish survival during transport. The findings highlight the transformative potential in optimizing fish transport for sustainable aquaculture. Refinement of nanobubble packaging and aeration could significantly increase survival rates and efficiency, promoting sustainable aquaculture practices.

Keywords: Live fish transport, nanobubble aeration, survival rates, sustainable practices

INTRODUCTION
The aquaculture industry in Nepal has been rapidly expanding in recent years, with a particular focus on the farming of trout in the hill regions of the country (Gurung 2008; Mulmi 2017). One of the major challenges faced by fish farmers is the high mortality rate of fingerlings during live transportation, often exceeding 50% due to factors such as low dissolved oxygen levels, accumulation of metabolic waste, and physical stress (Hargreaves and Steeby 1999). The live transport of fish presents several challenges that can compromise their health and survival. A primary concern is oxygen depletion in the water due to the respiration and bacterial activity of the fish, leading to stress, suffocation, and mortality (Guan et al. 2021). The accumulation of metabolic wastes such as ammonia and carbon dioxide create toxic conditions (Lekang 2013). Temperature fluctuations increase disease susceptibility and reduce appetite (Wedemeyer 1997). Physical injuries from improper handling, overcrowding, and poor water quality increase infection risks (Marino et al. 2016). Confined stressful conditions facilitate pathogen transmission between infected and healthy individuals (Hastein et al. 2005). Furthermore, the deterioration of water quality due to factors such as pH changes, ammonia, and the accumulation of organic matter negatively impact fish health (Lekang 2013). The combination of low oxygen, poor water quality, and injury induces significant stress, compromising immune function, growth, and survival rates (Wedemeyer 1997; Marino et al. 2016). Traditional transportation methods are limited by degradation of water quality over time, restricting safe transport distances (Guan et al. 2021), while conventional aeration is energy-intensive (Lekang 2013).

Nanobubbles (NB), gas bubbles ranging from a few nanometers to a few hundred nanometers in size (Edzwald 2010; Parmar and Majumder 2013), offer a potential solution for live fish transportation. Their high surface area-to-volume ratio enables efficient oxygen dissolution in water (Benstaali et al., 2013), crucial for meeting the respiratory needs to transport fish such as trout. Incorporating nanobubbles provides advantages such as improved oxygen transfer, longer duration of oxygen supply, reduced fish stress, and energy efficiency over traditional oxygenation methods (Hutagalung et al. 2023; Liu et al. 2016). Specialized nanobubble generators disperse nanobubbles in the water holding the fish, ensuring a continuous oxygenated supply during transit (Cheng et al. 2018; Yao et al. 2020). While ongoing research explores the optimal conditions and parameters for different species and scenarios (Guan et al. 2021; Shafiei et al. 2022), nanobubbles present a promising approach to address the challenges of live fish transportation.

The purpose of this study is to investigate the application of nanobubbles as an innovative approach to improve the conditions and survival rates of live trout during transportation. Nanobubbles are unique properties that may address several key challenges faced in live fish transportation. The findings could lead to improved practices and technologies that support the aquaculture industry by allowing long-distance transportation of live trout while minimizing losses and maintaining high fish health and quality. This introduction paragraph aims to provide an overview of the potential benefits of nanobubble technology in enhancing the survival rate of trout fingerlings during transportation in the context of Nepal's aquaculture industry.

MATERIALS AND METHODS

Study location

Pre-tests of aeration systems and fish packaging including conditioning were conducted at Trout Research Station, Dhunche (1900 masl), Rasuwa. The effectiveness of different aeration systems and gas diffusion in the transport of live trout fish was evaluated through round trip transport of fish from Dhunche to the lower part of Nuwakot district (600 m).

Experimental Design of Aeration System

This investigation focused primarily on evaluating the effectiveness of various aeration methods, specifically oxygen injection and diffuser systems, in maintaining adequate levels of dissolved oxygen to prevent hypoxia and improve the overall health of fish. This study used an EDON nanobubble generator and an ozone generator.

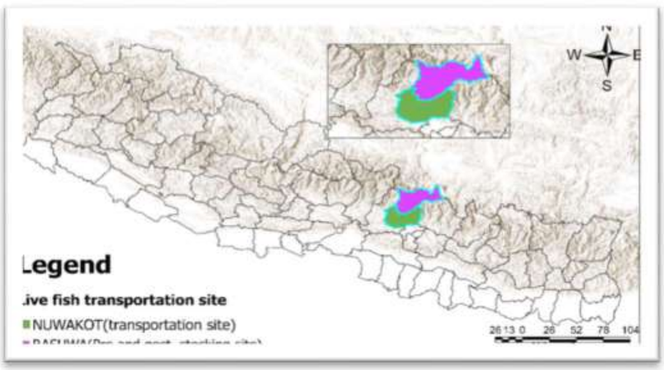

Figure : 1 Live the fish transport site of Nuwakot and Rasuwa

Closed system
In closed systems, trout fingerlings weighing 10 g each were stocked at two different densities: 500 and 750 fingerlings per plastic pack. These packs were subjected to various gas supply systems, including oxygenated water, oxygen nanobubble water, ozone nanobubble water, and air nanobubble water. The experiment followed a Factorial Completely Randomized Design (CRD) of 2 × 4 × 3 to assess the duration of survival under different gas supply conditions. A continuous dissolved oxygen (DO) sensor was placed inside each pack to monitor DO levels. The trial was terminated when the dissolved oxygen reached 4 mg/L due to the respiration of the fish.

Open system
In the open system, a 20-liter vessel housed 750 fingerlings for 8 h, with aeration methods including continuous infusion of four treatments: oxygen nanobubbles, normal air aeration, air nanobubble aeration, and ozone nanobubble aeration, after a 3-replication (CRD 4 × 3) design.

Transportation in an open system
In adapting transportation for open systems, a 100-liter vessel was utilized to house 3000 fingerlings for a duration of 12 hours. To ensure uninterrupted power supply during transportation, a separate electric backup generator was employed. This system implemented continuous aeration employing two treatments: oxygen nanobubbles and conventional oxygen aeration. The experiment followed a six-replication design with a completely randomized design (CRD) of 6 × 2. All vessels corresponding to the respective treatments were loaded onto a jeep/truck and transported from Dhunche to Trishuli and then back from Trishuli to Dhunche.

Sample/data collection
The process began by sampling the quality of the water to ensure precision. We recorded the number of trout fingerlings that were used in transportation and used a Pasco Scientific DO sensor meter for real-time monitoring with SparkVue using IOS software for water quality tracking factors such as temperature and dissolved oxygen. This approach focused on the well-being of fish and supported ongoing research. There is a carefully recorded time during the transportation of live fish when noting any changes in behavioral conditions are noted. As stress factors of ammonia and nitrite, the concentrations of transportation water were measured just before and after transportation.

Figure 2: Observation of survival and survival time with 500 fish per bag and 750 fish per bag in March 2022

Figure 3: Observation of the survival of trout fingerlings in an open tank fish transport system with oxygen aeration and nanobubble aeration in March 2022.

Figure 4: Observation of the survival of trout fingerlings in an open tank fish transport system with oxygen aeration and nanobubble aeration in March 2022.

Statistical analysis:

The differences between the group means of body weight gain, percent survival, survival time, loss, maintenance ratio, and water quality parameters were tested by analysis of variance (ANOVA). The post hoc test was applied to determine the significance of differences between the two means. All statistical tests were performed using the XLSTAT 2019 statistical package. Comparisons were made with 5% probability.

Instruments Validation and Quality Control

This study evaluated the performance of the EDON nanobubble generator, a specific nanobubble technology, at the Nepal Academy of Science and Technology (NAST) in Khumaltar, Lalitpur. To evaluate the efficiency of the generator, we employed a Zeta seizer for a comprehensive analysis of the nanobubbles produced. This assessment included measures of factors such as bubble size distribution, concentration, and stability over time.

Loss-maintain ratio

In the context of trout fish, the loss maintenance ratio refers to the efficiency of the transportation process in minimizing fish losses during transit. This ratio was calculated by comparing the number of fish initially loaded onto a transport vehicle or container with the number of fish that successfully reached their destination without perishing. The formula to calculate the loss maintenance ratio is as follows:

$$\text{Loss Maintain Ratio } = \frac{Total\ cost\ of\ loaded\ fish}{Cost\ of\ succesfully\ transported\ fish}\ x\ 100\%$$

In this formula:

The "Cost of Fish Successfully Transported" represents the number of loaded fish that reach their destination alive. The 'Total Cost of Fish Loaded' refers to the total quantity of fish initially loaded onto the transport vehicle. A higher loss maintenance ratio indicates better efficiency in ensuring safe and successful transportation of trout, which is crucial for maintaining profitability and economic viability.

RESULTS

In the study comparing closed packaging systems with different fish densities (500 fish/bag and 750 fish/bag), various parameters including survival rate, survival time, loss maintain ratio, DO levels, temperature, pH, ammonia and NO_2 concentrations were evaluated under different water conditions (normal water air NB water, oxygen NB water, and ozone NB water). For both fish densities, survival rates varied significantly among different water conditions 500 fish/bag: F 750 fish/bag: at $p < .05$. In the case of 500 fish/bag, oxygen, NB water showed the highest survival rates 99.6±0.1%, followed by ozone NB water, air NB water, and normal water. Similarly, for 750 fish/bag, oxygen NB water exhibited the highest survival rates 99.3±0.0%, followed by ozone NB water, air NB water, and normal water. Survival time also showed significant differences between conditions for both densities. Oxygen NB water consistently exhibited the longest survival time, followed by ozone NB water, air NB water, and normal water, regardless of fish density. Loss maintain ratio DO levels, temperature, pH, ammonia and NO_2 concentrations showed similar trends in different water conditions for both fish densities, with oxygen NB water generally providing the most favorable conditions, followed by ozone NB water, air NB water, and normal water.

Table 1 : Mean (± standard deviation) of survival rate, survival time, trout fingerlings, and water quality in plastic bags under the closed fish transport system in the Rainbow Trout Fishery Research Center, Dhunche Rasuwa.

Observations	Closed Packing system (500 fish/bag)			
	Normal water	Air NB water	Oxygen NB Water	Ozone NB water
Survival (%)	64.5±1.2^{a}	88.7±1.0^{b}	99.6±0.1^{c}	99.3±0.1^{c}
Survival time (min)	36.8±0.4^{a}	73.5±1.5^{b}	190.5±1.5^{d}	151.5±5.1^{c}
Loss Maintain Ratio (%)	57.4±1.4c	86.4±1.1b	99.5±0.1a	99.2±0.2a
DO (mg/L)	7.6±0.1^{a}	8.4±0.1^{b}	25.2±0.2^{d}	21.0±0.2^{c}
Temperature (^{o}C)	15.4±0.0^{a}	16.3±0.1^{b}	17.3±0.1^{c}	17.6±0.2^{c}
PH	7.5±0.0^{c}	6.8±0.0^{a}	6.7±0.1^{a}	7.1±0.0^{b}
Ammonia (mg/L)	0.38±0.05^{c}	0.10±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}
NO_2 (mg/L)	0.24±0.01^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
	Closed Packing system (750 fish/bag)			
Survival (%)	53.9±2.7^{a}	87.7±0.1^{b}	99.3±0.0^{c}	99.0±0.2^{c}
Survival time (min)	30.0±0.0^{a}	48.9±0.4^{b}	142.5±2.9^{d}	132.0±2.4^{c}
Loss Maintain Ratio (%)	44.7±3.2c	85.2±0.1b	99.1±0.1a	98.8±0.2a
DO (mg/L)	7.6±0.1^{a}	8.4±0.1^{b}	25.2±0.2^{d}	21.0±0.2^{c}
Temperature (^{O}C)	15.4±0.1^{a}	17.4±0.1^{b}	18.4±0.1^{c}	18.7±0.1^{d}
PH	7.4±0.0^{b}	6.6±0.1^{a}	6.6±0.1^{a}	6.6±0.2^{a}
Ammonia (mg/L)	0.45±0.03^{c}	0.10±0.00^{b}	0.10±0.00^{b}	0.00±0.00^{a}
NO_2 (mg/L)	0.24±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Note: Different superscript letters within the row are significantly different at α0.05.

In examining the open-live fish transportation performance of oxygen nanobubble (NB) water versus oxygen-aerated water for live fish transportation, several critical parameters were evaluated, shedding light on their respective effectiveness and implications for fish survival and water quality. The oxygen NB water demonstrated significantly higher survival rates 99.0±0.1% compared to oxygen aerated water 76.7±5.2%, emphasizing its potential as a superior transport medium to maintain fish viability. The oxygen NB water exhibited substantially higher initial concentrations 18.0±0.3 mg/L and final 13.0±0.2 mg/L DO compared to oxygen-aerated water 8.6±0.3 and 6.5±0.7 mg/L, respectively), indicating better oxygenation and potentially reduced risk of hypoxia during transport. Both oxygen NB water and oxygen aerated water maintained similar temperatures 18.0±0.1 and 16.8±0.3°C, respectively, suggesting a minimal impact on temperature regulation between the two treatments. The pH levels in the oxygen NB water pH 6.4±0.0 were slightly lower compared to the oxygen aerated water pH 6.5± 0.1, although the difference is relatively small and may have minimal physiological effects on the transported fish. The oxygen NB water showed lower NO_2 0.10±0.00 mg/L and ammonia 0.10±0.00 mg/L compared to oxygen-aerated water 0.23±0.01 mg/L for NO_2 and 0.20±0.04 mg/L for ammonia), indicating a potentially reduced accumulation of nitrogenous wastes and associated stress on the fish. The oxygen NB water exhibited a higher loss maintaining ratio 88.6±0.1% compared to oxygen aerated water 72.0±6.2%, indicating better maintenance of fish health and viability during transportation.

Table 2: Mean (± standard deviation) of survival rate, survival time, trout fingerlings and water quality in 20 L vessels under the open fish transport system at the Rainbow Trout Fishery Research Center, Dhunche, Rasuwa

Observation	Oxygen NB water	Oxygen-aerated water
Survival (%)	99.0±0.1[b]	76.7±5.2[a]
Initial DO (mg/L)	18.0±0.3[b]	8.6±0.3[a]
Final DO (mg/L)	13.0±0.2[b]	6.5±0.7[a]
Temperature (°C)	18.0±0.1	16.8±0.3
pH	6.4±0.0	6.5±0.8
NO_2 (mg/L)	0.10±00[a]	0.23±0.01[b]
Ammonia (mg/L)	0.10±00[a]	0.20±0.04[b]
Loss Maintain Ratio (%)	88.6±0.1[a]	72.0±6.2[b]

Note: Different superscript letters within the row are significantly different at α0.05.

The descriptive statistics compared with live fish transportation practices; oxygen nanobubbles, aerated water, and oxygen-aerated water for live fish transportation reveal notable differences across various parameters, providing insights into their effectiveness and implications for fish survival and water quality. The nanobubble water of open system demonstrated the highest survival rate 97.3±0.1%, followed by aerated water 90.3±1.0% and oxygen aerated water, indicating the potential benefits of oxygen nanobubbles in maintaining fish viability during transport (Table 3). The water exhibited the highest initial DO concentration 19.0±0.2 mg/L, followed by aerated water 9.6±0.1 mg/L and oxygen aerated water, suggesting superior oxygenation capacity in the water treated with nanobubbles. Similarly, water treated with oxygen nanobubbles maintained the highest final DO concentration 13.1±0.3 mg/L, followed by aerated water 7.1±0.2 mg/L and oxygen-aerated water, reinforcing its effectiveness in maintaining oxygen levels during transportation. Both the oxygen nanobubble treated water and oxygen-aerated water maintained similar temperatures 19.6±0.0 and 20.1±0.0 °C, respectively, indicating consistent thermal conditions in all treatments. Oxygen nanobubble-treated water exhibited a lower pH 6.5± 0.1compared to aerated water 7.2±0.0, suggesting potential differences in the water chemistry between the two treatments. The water displayed lower levels of NO_2 0.00±0.00 mg/L and ammonia (0.00±0.00 mg/L) compared to aerated water 0.02±0.00 mg/L for NO_2 and 0.01±0.00 mg/L for ammonia, indicating improved water quality and reduced accumulation of nitrogenous waste accumulation. Oxygen nanobubble-treated water demonstrated a higher loss maintenance ratio 96.8±0.1% compared to aerated water 88.4±1.2%, indicating better preservation of fish health and viability during transportation.

Table 3: Mean (± standard deviation) of survival rate, survival time, trout fingerlings, and water quality in 100L vessels with open fish transported from Dhunche to Trishuli, and then back from Trishuli to Dhunche

Descriptive	Oxygen NB water	Oxygen-aerated water
Survival (%)	97.3±0.1[b]	90.3±1.0[a]
Initial DO (mg/L)	19.0±0.2[b]	9.6±0.1[a]
Final DO (mg/L)	13.1±0.3[b]	7.1±0.2[a]
Temperature (°C)	19.6±0.0	20.1±0.0
pH	6.5±0.1[a]	7.2±0.0[b]
NO_2 (mg/L)	0.00±0.00[a]	0.02±0.00[b]
Ammonia (mg/L)	0.00±0.00[a]	0.01±0.00[b]
Loss Maintain Ratio (%)	96.8±0.1[b]	88.4±1.2[a]

Note: Different superscript letters within the row are significantly different at α0.05.

DISCUSSION

The present study underscores the critical importance of water quality in closed packing systems for fish, with profound implications for their survival rates, longevity and overall well-being (Ahmed et al. 2023; Ashley 2007; Miranda-Filho 2019; Hoseini et al. 2022; Joshy et al. 2022; Kamalam et al. 2017; Owen 2023; Shabani et al. 2016; Singh et al. 2004). Irrespective of fish density (500 fish/bag or 750 fish/bag), oxygen nanobubble (NB) water consistently emerged as the most favorable environment, facilitating higher survival rates and extended survival times. This finding highlights the crucial role of maintaining optimal dissolved oxygen levels in aquaculture settings, corroborating previous research (Hao 2021; Mahasri et al. 2018).

Furthermore, the study delineates a distinct hierarchy among the tested water conditions, with oxygen NB water demonstrating superior conditions, followed by ozone NB water air NB water, and normal water. The observed trends in the loss maintenance ratio and other water quality parameters, such as lower pH levels, NO_2 and ammonia concentrations, corroborate the superiority of oxygen NB water in preserving fish health and viability (Hao 2021; Owen 2023). In particular, although increasing fish density resulted in lower survival rates, shortened survival times, and compromised water quality under all conditions, the relative ranking among water conditions remained consistent. This observation aligns with previous findings that highlight the detrimental effects of high stocking density on fish welfare during transportation (Kamalam et al. 2017; Muzaddadi et al. 2017; Singh et al. 2004).

The study findings underscore the paramount importance of vigilant water quality management to ensure the prosperity and sustainability of closed packing systems in aquaculture operations. Specifically, the superior performance of oxygen NB water compared to oxygen-aerated water and aerated water in maintaining fish viability during transportation is a significant outcome (Joshy et al. 2022; Lima et al. 2020; Suryadi et al. 2020). This conclusion is supported by higher survival rates, better oxygenation indicated by higher dissolved oxygen concentrations, lower concentrations of nitrogenous wastes, and a higher loss maintaining ratio observed in NB oxygen water treatment (Barton and Peter, 1982; Miranda-Filho 2019; Hao 2021; Sadek and Ching 2019).

The study's findings suggest that oxygen NB technology holds promise for enhancing live fish transportation outcomes by improving survival rates, maintaining optimal water quality, and minimizing stress on transported fish (Ahmed et al. 2023; Ashley 2007; (Barton and Peter, 1982; Miranda-Filho 2019; Hoseini et al. 2022; Joshy et al. 2022; Kamalam et al. 2017; Owen 2023). Further research and practical application of this technology could contribute to advances in aquaculture and fisheries management practices, addressing the challenges associated with ensuring fish survival and well-being during transportation (Ashley 2007).

Furthermore, the study emphasizes the importance of considering the duration of transport based on the packing system and examining how stock density, water quality, and packing systems affect vital water parameters such as dissolved oxygen, temperature, pH, and ammonia levels (Hao 2021; Owen 2023). These factors interact and can lead to stress, weakness of the immune system, and oxygen shortage, ultimately affecting fish survival (Kamalam et al. 2017; Owen 2023). The longer transport times of the oxygen NB water system can contribute to improving the endurance of the fish, as supported by previous findings (Lima et al. 2020; Suryadi et al. 2020). The integration of sensors and communication protocols for online monitoring of oxygen levels in tanks has proven beneficial in addressing mortality rates and providing early warning systems for oxygen management during fish transport (Zhang et al. 2020; 2021; 2023). These innovations have transformed aquaculture by improving the survival of fish during transit and enabling informed decision making by fish farmers to reduce aquaculture mortality.

CONCLUSION

The study emphasizes the crucial role of water quality in fish transport and closed packing systems. It highlights oxygen NB water as the most beneficial environment for fish survival and well-being, surpassing other water conditions such as ozone NB water, air NB water, and normal water. The high stock density negatively impacts fish welfare during transportation, necessitating careful water quality management. The oxygen NB water outperforms other aeration methods in maintaining fish viability during transit, indicating its potential to improve live fish transportation outcomes. The integration of sensors for online monitoring of oxygen levels in tanks proves beneficial in reducing mortality rates during transport. The study suggests that nanobubble aeration could be a more effective alternative to traditional methods, promising to improve fish welfare and survival rates during transit in aquaculture practices.

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