

APPLICATIONS OF NANOBUBBLE AERATION TECHNOLOGY FOR AQUACULTURE PRACTICES: A REVIEW

Hare Ram Devkota^{1,2,3*}, Dilip Kumar Jha¹, Tista Prasai Joshi²,
Shreemat Shrestha³

¹ Agriculture and Forestry University, Rampur, Chitwan, Nepal

² Nepal Academy of Science and Technology, Lalitpur, Nepal

³ Nepal Agricultural Research Council, Lalitpur, Nepal

Corresponding author * Email: hdevkota6@gmail.com

ABSTRACT

Notably, there has been widespread interest in the multiple possible uses of nanobubbles (NB) with a diameter of less than 100 nm. The introduction of NB technology in Japan, the United States, Europe, and other countries shows that it has enormous potential, even in the early stages of exploration in various industries. However, the full extent of its impact on aquaculture is not yet fully understood. Nanobubbles have shown promising results in improving water quality in aquaculture systems, increasing oxygen levels, and promoting fish health. However, further research and experiments are needed to determine the optimal conditions for their application and to fully understand their long-term effects on fish growth and overall production efficiency. Not only fish productivity highly positively correlated with dissolved oxygen (DO) concentration in the aquatic environment but also the size of the bubbles is inversely related to the solubility and stability of DO. Therefore, it plays a crucial role in this interdependence. However, it is worth noting that existing aquaculture practices often unknown the importance of oxygen nanobubbles. These nanobubbles can be quickly generated and distributed on the water surface using various devices, creating millions of oxygens nanobubbles in a limited volume of water to have a significant impact on the regulation of aquatic environments. Using nanobubbles in aeration technology is a promising option for the blue revolution in aquaculture practices. The article offers a comprehensive overview, providing valuable insights into the significance and potential benefits of NB technology in the aquaculture sector.

Keywords: Aeration, Aquaculture, Dissolved Oxygen and Oxygen Nanobubble

INTRODUCTION

Aquaculture plays a crucial role in the global fisheries industry. Intensive aquaculture aims to provide agricultural fish with essential resources, including access to quality water and sufficient food. The primary focus was identifying and prioritizing the key factors of DO that played a significant role in aquaculture development. According to Mallya, (2007), low dissolved oxygen levels can pose a hazard and impact the water's ability to sustain biological agents. Consequently, a large focus has been placed on research to increase dissolved oxygen levels in water. Numerous factors, such as their diet and water quality, affect the health of aquatic organisms, where dissolved oxygen governs the overall health of aquatic animals' DO concentration (Guo et al., 2011). Additionally, studies have shown that higher concentrations of DO can increase these organisms' growth rates (Boyd, 2017). Numerous studies are underway to enhance water quality metrics by implementing different aeration techniques. Rognerud et al. (2020) have recently improved the solubility and stability of DO. A range of biological and non-biological activities influence the solubility of substances in an aquatic environment. These activities, such as respiration, photosynthesis, and diffusion, contribute to DO solubility, which is dynamic (Sarkar et al., 2022). The solubility of gases is also influenced by water temperature, atmospheric air, and water pressure, which are interconnected and maintained in equilibrium in nature (Heinemann & Weinhardt, 2004 ; Meegoda et al., 2018). Consequently, over time, the DO levels fall below the anticipated range, resulting in severe consequences for the aquatic ecosystem. The fact that innovative progress has been made in water aeration technology indicates that efforts have been made to address the anoxic condition of the pond. The prevailing belief is that 70% of the gas injected into water is diffused out of the water into the atmosphere. According to Suryadi et al., (2020), active aeration produces more microbubbles than passive aeration, where the research shows that passive aeration methods make 20% of microporous tubes, 25% of porous stone, and 44% of ceramic diffusers dissolve gas in water. On the other hand, active methods demonstrate higher gas solubility, with 60% achieved through the venturi nozzle and 80% through the oxygenation cone.

Creating the first microbubbles (MBs) in Japan involves a turbulent mixture. The size fluctuation of the bubbles showed a discrepancy, with a microbubble measuring approximately 200 nm (Akimi, 2017). This microbubble also exhibited distinct physical characteristics of solubility and stability (Agarwal et al., 2011). The International Organization for Standardization (ISO) has defined the size of

bubbles as less than 100 μ m in diameter, commonly referred to as fine bubbles (Hata et al., 2019). The primary objective of the inaugural International Symposium on NB was to implement NB technology to enhance aquaculture production (Akimi, 2017). NB exhibits distinctive advantageous characteristics that result in the generation of hydroxyl radicals. These radicals, including H⁺, OH⁻, and H₂O₂, possess high reactivity and are crucial in eliminating bond impurities. Moreover, NB aids in enhancing fish population density without compromising the quality of water. Additionally, it helps mitigate the harmful effects of pathogens in fish (Mead et al., 1976). The dissolving performance of NB is more effective than that of ordinary gas bubbles. According to Beijnen Yan (2021), the oxygen dissolving efficiency in the NB is 85 percent, which is significantly higher than that of conventional aeration by over three times. According to Mahasri et al. (2018), NB generators experienced increased DO levels of up to 25 ppm from an initial level of 6.5 ppm. The performance of NB aeration is significantly superior to that of other standard aeration methods. According to Gilmour Zimmerman, (2020), a traditional surface aeration system generates sludge and disperses harmful gases into water columns, producing oxidation of the surface water. The technology has distinct characteristics that contribute to the overall health and productivity of the pond ecosystem. According to Meegoda et al., (2018) and Batagoda et al., (2019), high oxygen levels at the bottom are crucial in reducing toxicity through oxidation. This process converts organic sediments and waste into fish feed, enhancing fish productivity and accelerating growth. The lack of understanding about the unique beneficial properties of NB has prevented it from becoming a critical area. The usefulness of this property extends to various fields such as chemistry, physics, agriculture, medicine, the environment, and industry. The paper comprehensively analyzes the characteristics, manufacturing, and use of NBs in aquaculture. It examines their impact on fish growth, production, live fish transportation, and disease treatment, as depicted in Figures 1 and 2.

CHARACTERISTICS OF NB

The unique properties of the NB can be attributed to its small size. According to the ISO, the recommended size limit is 100 μ m. There is a positive correlation between the size of gas bubbles and the dissolution rate when the merging of bubbles increases in size. A decrease in surface area and velocity coincides with this, which eventually causes the bubbles to rise to the surface and burst. Bernoulli (1738; Hoare & Pelton (2007) observed and studied this phenomenon. when the small-sized bubble undergoes Brownian motion and eventually bursts within the water column. This process leads to the formation of NB and makes the bubble soluble. The high interior pressure and negative potential of NBs contribute to the

acceleration of gas dissolution (Meegoda et al., 2018). The explosion of a microbubble (MB) converts into a nanobubble (NB) with specific properties, where a gas bubble containing an oxygen molecule moves slowly and is dissolved in a liquid. This dissolved form is characterized by a negative zeta potential (ZP). According to Michailidi et al. (2020), one of the notable characteristics of NBs is that the particles undergo collisions for extended periods while remaining neutrally buoyant in a water column. In addition, it is worth noting that the internal pressure of liquid NBs is higher than the surrounding pressure. Ljunggren Eriksson (1997) observed a particular characteristic that accelerates the process of gas dissolution in liquid. According to Kundu et al. (2018), the zero point size of nanobubbles tends to decrease solubility over time due to the increase in size and Brownian motion. Based on the experimental findings, it can be concluded that ZP exhibits a negative correlation with salinity, bubble size, and temperature. The size of the bubbles remains unchanged regardless of the temperature of the solution. According to Meegoda et al. (2018), more minor, stable bubbles with higher ZP are observed with higher pH levels and OH ion concentrations. Cerron-Calle et al. (2022) found that NB demonstrates stability in high pH ranges of 5–6 and 8–9. As the negative ZP value increases, there is a corresponding increase in the medium's acidity. This increase in acidity leads to an increase in bubble size, which causes the bubbles to become unstable. Figure 1 illustrates the comprehensive concept of bubble classification, encompassing various factors such as size, formation, dissolution, and stability mechanisms.

NB Production System

The NB production system relies heavily on the pressure and velocity of the liquid. Bernoulli (1738) and Beijnen Yan (2021) describe the velocity of the gas, the internal pressure of the gas, and the bubble relationship when the velocity increases, the internal pressure decreases. This relationship is particularly relevant in the production of NBs, where the pressure of a liquid or gas is often altered through the cavitation process, as explained by (Agarwal et al., 2011). The NB production system can be categorized into three main processes: pressure, dissolution, and electrolysis. Gas-liquid and wave-pressure methods are widely employed to observe the cavitation action. The gas-liquid method is a process that entails the introduction of a substantial pressure of either a liquid or gas into a gas-liquid medium to produce NB. The production methods for NB are in detail below.

Gas-liquid pressure method

Commercial NB generators utilizing the Bernoulli principle are available on the market. According to Minible (2020), the MiniBle series faucet aerator produces

a significant number of 100-nm-sized NBs, specifically 600 million per square cm of liquid pressure ranging from 2 to 3.5 bars. High-pressure liquid on the nozzle creates a suction effect that draws the gas through the flow path. This process forms approximately 90% of the bubbles, which appear milky white-water color (Samuel & Power, 2020). The NB-generated water pump is widely recognized as a popular and frequently utilized instrument. According to Hideki (2014), milky white MB and NBs are emitted by absorbing atmospheric gas, with an average emission of 50 nm of MB.

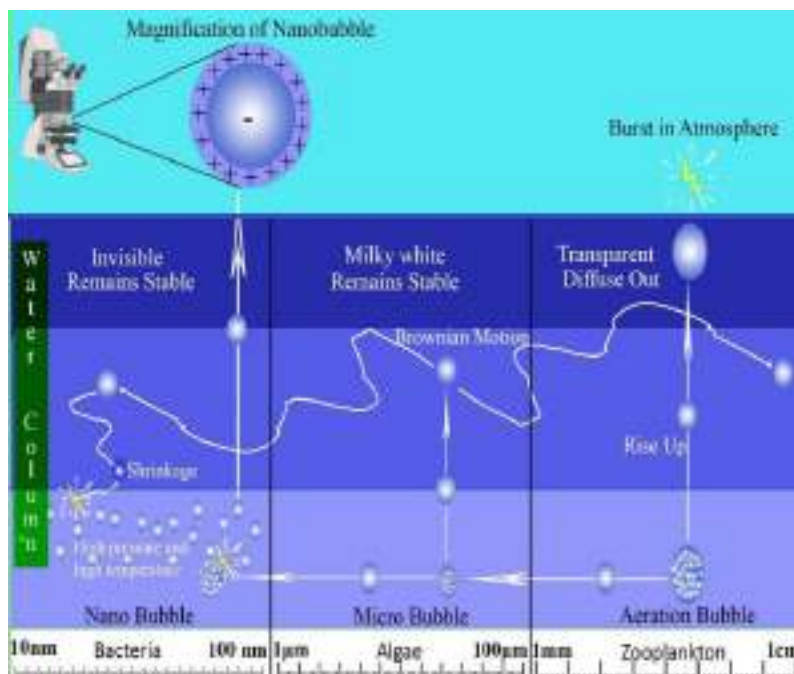


Figure 1. A conceptual overview of different bubbles' classification, stability, solubility, and surface area

The carbon-ceramic porous membrane is a low-pressure working product that applies gas pressure to the liquid. Numerous studies have been carried out to explore the commercialization of NB characterization by using different instruments. NB was characterized using ozone gas in the spiral liquid flow method. The zeta potential (ZP) was measured to be -21, and the initial bubble

size was 247 nm at a concentration of 4.55×10^{10} . Additionally, oxygen gas was used in the venturi nozzle, resulting in a zeta potential of -4.3 and an initial bubble size of 344 nm. These measurements were taken over seven days (Hu & Xia, 2018). A separate study was performed to achieve the same objective, where the ZP was found to be -20. The initial size of the bubbles ranged from 150 to 200 nm, with a concentration of 4.0×10^9 . Etchepare et al. (2017) observed that these bubbles remained stable in water for 60 days. The study utilized air in a splitter-type ejector with a ZP of -20 and an initial bubble size ranging from 700 to 900 nm. According to Cerron-Calle et al. (2022), the ZP at the end of the experiment was measured to be -25. Additionally, the initial bubble size was reported to be within the range of 350–400 nm. The instruments play a significant role in producing stable NBs for commercial purposes, ensuring long-term gas stability in water

Wave Pressure Method

Ultrasound has been found to generate NB that can physically and chemically impact aquatic and water microorganisms. According to Dong et al. (2012), ultrasonic transducers can generate oxygen molecules in water through the compression and rarefaction of acoustic waves. This process causes the covalent bond of water to break, forming tiny gas bubbles containing hydroxyl free radicals. Hydroxyl radicals, being highly reactive, can function as an antipathogen (Uchida et al., 2018). Commercial ultrasonic NB technology, commonly called an ultrasonic algae killer, is prevalent in eradicating algae. According to Cho et al. (2005), when subjected to ultrasound, NB's zeta potential (ZP) ranged from -2 to -28. Additionally, the initial bubble size was reported to be 249 nm, and this size remained unchanged in water for 60 days. Recently, green technology has gained recognition for its environmentally friendly nature, primarily due to its lack of chemical contamination.

Electrolysis

The principles of electrolysis have been well-established for over two centuries. Electrolysis is a straightforward process that involves immersing the anode and cathode in water and applying a minimum direct current voltage (DC). The presence of an electrolyte is unnecessary; the minerals found in water as a conductivity parameter, serve as electrolytes, facilitating the production of bubbles. However, the issue of anode corrosion poses a significant challenge. The selection of corrosion-resistant materials, including titanium, cadmium, and steel, is necessary. According to Hideki (2014), this method can produce micro- or NBs of hydrogen and oxygen, with average sizes varying between 35 and 100nm. The volume of gas produced in this process is a ratio of 2 parts hydrogen to 1 part

oxygen. The current application of this method involves the production of hydrogen fuel, resulting in the generation of oxygen as a by-product. Electrolysis is employed to effectively isolate minuscule hydrogen and oxygen molecules generated by a commercially available product utilized in the aquarium for ornamental fish. The utilization of this method results in an increase of over 50% in DO compared to conventional methods. The use of this option in hydroponics, aquaponics, and ornamental fish farming sectors is a subject of debate and disagreement.

NB measurement technology

Particle size analysis instruments are utilized to perform quantitative measurements using different principles. The text describes different approaches to illustrating bubble measurement methods based on their characteristics. The absorption or transmittance of light on the NB is measured using a dynamic light scattering method. This method is used to analyze the parameters of the bubble formed in deionized water and electrolyte solution, as Xiaotong et al. (2022) described. In the microscope equipped with a video camera, the characteristics of the NB were analyzed using laser light and light diffraction methods. Specifically, a 525 nm laser light deflected the light that passed through the sample (Etchepare et al., 2017). The installed camera measured the quantity of diffracted light coming from the NB. The study utilized a different technique for tracking bubbles, explicitly focusing on 7-9 Brownian-moving bubbles within a given volume of liquid. This method assessed the bubbles' quality and quantity (Ferraro et al., 2020). To quantify NB in its natural environment, the researchers implemented electrical detection and image analysis techniques for characterizing NB (Marui, 2013). In the present era, scientific instruments are developed based on light absorption or transmittance principles.

NB for next-generation aquaculture

Aquaculture is an industry experiencing significant growth, playing a crucial role in meeting the increasing demand for animal protein. In recent days, several issues have emerged, including concerns about disease sensitivity and the safe transportation of products from farms to consumers. Due to the issues above, numerous countries are actively searching for a comprehensive solution. The introduction of NB technology into the fisheries sector is a recent development aimed at addressing the issue. Although the technology is still in its early stages, several countries have already implemented it in various ways within the aquaculture industry. Samuel & Power (2020) state that several countries, including America, Holland, the United Kingdom, Australia, and Japan, utilize it

for various purposes, such as lakes, aquaria, and swimming pool cleaning. Additionally, it is used in the production of prawns, oysters, and spirulina, as well as fish food processing (Phan et al., 2020), wastewater treatment (Li et al., 2023), and irrigation (Minamikawa et al., 2015). The utilization of this technology in India extends to salmon farming and the transportation of salmon fry. Figure 2 provides an example of aquaculture utilizing NB technology.



Figure 2. An overview of NB production methods and the aquaculture application sector of aquaculture

NB used in Fish Culture

Multiple studies have examined different aeration systems used in intensive fish culture. According to James Tidwell (2012), the profitability of the recirculating aquaculture system (RAS) is twice that of traditional aerated pond fish farming (Suryadi et al., 2020). They conducted a study comparing venturi-produced MBs to a standard aeration system to assess the growth performance of tilapia fry at different stocking densities in 15 liters of water. The study found that the growth performance in the venturi system was significantly higher than in the normal aeration system. It has also examined how well the water exchange system, the RAS system, and a mix of the RAS and NB systems produced fish in young groupers, with 600 fish per cubic meter of water. The results indicated that combining RAS and NB treatments improves growth performance and productivity. In a related study, Vannamei shrimp were subjected to an experiment for 56 days. The experiment assessed the economic feasibility of two aeration systems: NB and non-NB. The shrimp were stocked at a density of 400

cubic meters. The results showed that the NB aeration system had a survival rate of 92%, an internal rate of return (IRR) of 18%, and a benefit-cost (B/C) ratio of 1.26. According to Mauladani et al. (2020), it is evident that an NB system is more feasible than a non-NB system. Another study was conducted in 50-m² ponds with 34,000 juvenile white-leg shrimp at a stocking density of 680 m³ for 81 days. The findings indicated that the NB treatment resulted in better outcomes than diffuser-type aeration. Specifically, the NB treatment showed a higher survival rate, improved feed conversion ratio, increased total harvest, and enhanced productivity. Additionally, the NB aeration method led to a reduction in total pathogens. Notably, the total harvest and productivity nearly doubled with NB aeration (Rahmawati et al., 2020). The NB aeration system demonstrates higher fish productivity compared to other aeration systems.

Use of NB in live fish transport

Two fish transport methods exist: closed and open. Practical applications use polythene bags with oxygen and water tanks with supplied oxygen or air. Indian major carp and Chinese carp weighing 300–1500 g was traditionally aerated for 2–6 hours. A 600-700 kg distance group and a 50-100 kg distance group received the fish. Long-distance mortality was 100%. found that moving live fish without an appropriate aeration system caused the problem Muzaddadi et al. (2017). The experiment examined Pirarucu (*Arapaima gigas*) fingerlings for six hours using physiological and water quality tests. One group of fingerlings was placed in 170 g/L plastic bags with NB oxygen supplements. The other group received normal water in 50 g/L plastic bags. We measured water quality before and after transportation. After transport, water temperature, dissolved oxygen, carbon dioxide, and non-ionized ammonia increased, but pH decreased. Cortisol levels remained stable in the study. Fish in normal water did not die. NB oxygen supplements caused a 33% mortality rate and a three-fold increase in stocking density. found more fish per unit of NB water. This study examined the effectiveness of air blower, air NB, and oxygen NB at 100/L, 166/L, and 250/L stocking densities over 10 hours at 26 °C. The comparison between an air blower and oxygen showed that oxygen had different effects. NB transports more fish per liter than the average, increasing its efficiency fourfold Lima et al. (2020). Thongdon et al. (2019) experimented with investigating the dissolved carbon dioxide and oxygen levels in a chicken grunt (*Parapristipoma trilineatum*) over 30 minutes at 20 °C. The fish were subjected to sedation, causing a transition in their respiratory system from branchial to cellular. This transition was a result of the lethal effects of CO₂. During 22 hours, oxygen was transferred from water to tissue to regulate cellular respiration. After 22 hours, the fish are immersed in NB

water rich in oxygen for 2-3 hours. Carbon dioxide nanobubble was given as anesthesia and regained full consciousness after the end of the effects of the anesthetic when it was stored in a fully saturated oxygen nanobubble. CO₂ is a suitable and safe anesthetic for transporting fish over long distances at average water temperatures. A study was conducted in the sturgeon muscle to assess the effectiveness of oxygen, nitrogen oxide, ice, and regular water for long-distance live transport. A group of fish was divided into two conditions: one group was placed in pretreated NB water, which is considered desirable, while the other group was placed in regular water. In a programmable cooling system, the water was intentionally cooled to 0 °C. The fish then put its produced ice into the icebox. A separate water group was transported at a temperature of approximately 19°C. The total duration of transportation for both entities is 36 hours. The mortality rates for the NB and standard groups were 3.2% and 11%, respectively. The weight of the fish in the nanobubble group exceeds that of the water transported (Zhang et al. 2019). Table 1 provides a comprehensive overview of the process and data involved.

NB in Fish Health Management

Ozone NB has shown promise in aquaculture systems for increasing DO levels and lowering bacterial levels. NB-O₃ affected immune gene expression in Nile tilapia (*Oreochromis niloticus*), comparing fish that were treated and fish that were not treated. According to the findings, NB-O₃ has the potential to be effective in preventing pathogenic bacterial infections Linh et al. (2021). The Jhunkeaw et al. (2020) experiment aimed to investigate the potential infection of Nile tilapia with harmful pathogens. The participants were split into two groups based on the treatment they received. One group got NB, ozone, or the standard treatment in 50 L of water for 10 minutes. The size of NB was 130 nm, which is equal to about $2-3 \times 10^7$ O₃ bubbles/ml of concentration, and the oxidation-reduction potential (ORP) was 834 mV. The other group did not receive any treatment. According to the study, the group treated with NB had a significantly lower bacterial count, with a reduction of 99.29%. According to the test results, the NB-O₃ technology shows promise in suppressing dangerous bacteria and enhancing dissolved oxygen levels for fish health management. Both studies have demonstrated that microbubbles and nanobubbles hurt microorganisms. the study shows that the varying reactions observed have both positive effects on long-drive organisms Jhunkeaw et al. (2021).

Impact of NB on Fish Physiology

The respiratory function of aquatic species is crucial in determining their physiological state. According to Temesgen et al. (2017), aquatic organisms

engage in cellular and branchial respiration when the size of NB in the water is below 0.2 micrometers. Despite the significantly smaller skin pores (Inatsu et al., 2011), they can still diffuse from the skin to the underlying tissue. Tissues absorb significant quantities of NB to sustain cellular and branchial respiration. The lack of DO in water leads to the release of the catecholamine stress hormone in cells, resulting in elevated plasma cortisol levels in fish (Sumpter & J., 1997). According to Lewis & Lee (2007), the increase in heart rate and gill function occurs at a faster rate than is typically expected. In this situation, branchial breathing is often inadequate to meet the body's oxygen requirements. The relationship between stress feed efficiency and feed conversion ratio is crucial for proper feed assimilation (Afridha, 2021). The behavior of an NB under extreme conditions of high temperature (5000K) and pressure (1000 atm) is characterized by explosive bursting. The increase in temperature of the NB inside the tissue positively impacts the expansion of blood vessels. This expansion allows for greater oxygen flow to the gills, which is essential for their proper physiological functioning. Dual respiration, blood purification, and excretion or neutralization of toxic materials produced in the body play a crucial role in maintaining the physiological health of fish through dual respiration, blood purification, and excretion or neutralization of toxic materials produced in the body. The impact of NB technology on the physiological is positive (Akimi, 2017).

CONCLUSION

Aquaculture is poised to benefit significantly from nanobubble (NB) aeration technology, as explored in this comprehensive overview. The first part of the discussion looks at NB properties, how they are made, and what they can be used for, focusing on how much more gas- and solvent-stable they are than regular bubbles. Factors influencing NB size, formation, dissolution, and stability, such as pressure, temperature, pH, and zeta potential, are elucidated. The paper then delves into the potential advantages of NBs for aquatic organisms, summarizing studies comparing their performance with other aeration systems across diverse species, stocking densities, and water quality conditions. We also look at how NBs affect the breathing of fish cells and branches, the cleaning of their blood, and their stress hormone levels. We focus on how NB-O₃ technology can stop pathogenic bacteria. It is important to note that changing regular bubbles to NBs by changing existing aeration instruments is discussed. This shows how microbubbles (MB) and NBs differ regarding how stable and soluble they are in gas. Gas dissolution performance is inversely proportional to bubble size, solubility, and stability. In conclusion, NB aeration technology emerges as a promising solution to address low oxygen content in aquaculture water, offering benefits for fish culture, live fish transport, and fish health management.

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Table 1. Examples of the contribution of NB to live fish transportation and water quality management

Species	Condition	SD	Water quality					Mortality (%)	Before transportation		After transportation		Time (hour)	Reference
			DO (mg/L)	NH ₃ (mg/L)	CO ₂ (mg/L)	Temp (°C)	pH							
Juvenile pirarucu	Normal water	50g/L	5.6	0.05	2	27.8	7	0	4.45 (C)	41.9 (G)	7.67 (C)	104.6 (G)	6	Lima et al., 2020
	NB water	170g/L	15	1.34	12	31.42	6	33	2.33 (C)	44.18 (G)	15.11 (C)	132.13 (G)	6	
Sturgeon muscle	Normal water	NA	8.8	NA	NA	19.1	7.9	11.1	NA	5 (G)	NA	100 (G)	6	Zhang et al., 2019
	NB waterless	NA	91.20%	NA	3.1	1.3	7.2	3.2	19.3 (P)	4.7 (F)	19 (P)	4.7 (F)	36	
Tilapia fry	Air blower	100 (no/L)	4.8	0.12	NA	26.8	6.1	4.8	NA	NA	NA	NA	10	Thongdon et al., 2019
	Air NB	166 (no/L)	25	0.4	NA	26.9	7.7	7.6	NA	NA	NA	NA	10	
	Oxygen NB	250 (no/L)	11	0.3	NA	26.10	6-6.4	2.5	NA	NA	NA	NA	10	

Note: SD = stock density, G = blood glucose level, C = blood cortisol level, P = protein (%), F = fat (%), NA = not available

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