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Research

Photosynthetic Bacteria as an Alternative Wastewater Treatment in Freshwater Aquarium Fish Set Up

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ABSTRACT

Waste produced from aquaculture ultimately hampered the water quality and growth performance of species cultured. Therefore, the potential exploitation of photosynthetic bacteria (PSB) from aquaculture waste was investigated to treat the wastewater in the fish culture. In this study, the wastewater was collected from a fishpond and cultured in sunlight-exposed sterile bottles for 14 days. In the water additive experiment, five treatments (in a 30 cm^3 aquarium) were prepared namely cultured PSB (T1), aquatic plant hornwort (T2), aquatic plant salvinia (T3), positive control (P), and negative control (N). Five individuals of *Hyphessobrycon herbertaxelrodi* (2.5±0.5 cm length) were placed in each treatment and reared for 30 days. Meanwhile, for the wastewater treatment, four treatments (in a 30 cm^3 aquarium) were prepared namely wastewater from the hatchery fishpond (S1), wastewater from the hatchery fish tank (S2), positive control (P), and negative control (N). The results obtained indicate that T1 treatment significantly improved and maintained the water quality as compared to other treatments. The results showed the amount of ammonia and nitrite in S1 and S2 supplied with T1 for 30 days gradually decreased from day 1 until day 18. While zero amount of ammonia and nitrate was acquired from day 21 until day 30 of the wastewater experiment. The treatment with PSB showcased the bacteria's ability to utilize and absorb nutrients, thereby maintaining and improving water quality. The potential use of beneficial bacteria in the culture system can accelerate the nitrogen cycle for a sustainable way of wastewater management.

Key words: Alternative treatment, cultivation, supplement, sustainable water quality

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INTRODUCTION

Aquatic ecosystems, especially those employed for rearing freshwater fish in aquariums, face challenges related to the accumulation of nitrogenous compounds and pollutants arising from wastes and uneaten feed. Fish exhibit a remarkable ability to assimilate approximately 25% (Neil., 2019) of the nitrogen content in their food for metabolic requirements, while the remaining nitrogen is discharged as ammonium nitrogen, dissolved organic matter, or feces. The breakdown of uneaten feed and wastes by microbial activity results in the release of additional ammonium, indirectly contributing to water pollution and posing a threat that can cause the sudden death of fish (Zhang *et al*., 2018). Regular water change is a widely employed practice aimed at improving water quality in confined aquatic ecosystems. However, its practical efficiency may vary depending on specific circumstances. Furthermore, studies have indicated that frequent water replacement can play a significant role in the dissemination of fish diseases from infected areas to otherwise healthy ponds. Particularly concerning is the potential rise of unfavorable conditions, such as the activation of specific diseases like white spots, during periods of rainy weather when water temperature drops (Yang *et al*., 2018).

Nitrogen molecules such as ammonium and nitrite can pose a threat to fish when present in significant concentrations. Therefore, it is crucial to implement a range of measures to improve aquaculture waste management and ensure the sustainability of aquacultural practices. In China, the use of photosynthetic bacteria (PSB) has gained prominence in fish and shellfish hatcheries, farms, and wastewater treatment, aligning with the increasing need for environmentally sustainable aquaculture practices (Cao *et al*., 2020). Photosynthetic bacteria are widely distributed in natural environments such as soil, lakes, and oceans. They exhibit phototrophic growth, utilizing light as an energy source, and organic carbon compounds, sulfides, or hydrogen donors to fix carbon dioxide for anoxygenic photosynthesis (Chen *et al*., 2020). PSBs have the unique ability to simultaneously utilize various organic substrates and nitrogenous substances, contributing to the growth rate of fish. According to Zhang *et.* al., (2014) PSB manages to improve water quality as it changes the microbial community structure. Understanding the composition of microbial communities is crucial for the design of efficient wastewater treatment systems and contributes to the development of microbial ecology theories. By harnessing the capabilities of PSB, it is possible to improve water quality and enhance the integrity of microbial community composition in integrated multitrophic aquaculture systems (Ying *et al*., 2020). The utilization of PSB in wastewater treatment holds promise for achieving the recovery of nutrients and energy, as well as the degradation of refractory substances (Cao *et al*., 2020).

Nitrate accumulates through nitrification in fish culture systems. Therefore, it is vital to minimize excessive nitrate amounts to prevent potential nitrite accumulation and address environmental constraints related to the discharge of nitrate-rich effluent waters (Byod & Tucker, 2014). PSB are being added to freshwater fish aquariums to maintain the water quality for fish reared in aquariums over the long term and PSB helps decompose toxic substances like ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen thereby reducing their concentrations (Ringo *et al*., 2022). The using of PSB can help expand the colony of beneficial bacteria in a new aquarium habitat, hence accelerating the nitrogen cycle. As an alternative to typical aquarium fish systems, high-density water reuse systems with extremely low water exchange ratios are utilized (Ying *et al*., 2020).

Therefore, we hypothesized that the use of PSB in freshwater fish aquariums can contribute to sustainable wastewater treatment, reduce nitrogenous substances like ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen, and improve water quality for fish reared in the aquariums. PSB can decompose toxic substances and fix carbon dioxide for anoxygenic photosynthesis, potentially accelerating the nitrogen cycle and enhancing water purification in fish farming. Hence, the objective of this study is to evaluate the potential of using PSB as an alternative treatment to enhance waste management in freshwater Tetra fish aquarium setups. In this study, PSB from aquaculture waste was isolated and implemented as an alternative treatment to evaluate its potential to meet sustainable wastewater treatment in freshwater Tetra fish in the aquarium setup. Apart from that to further investigate the efficacy of the isolated PSB in treating wastewater, aquaculture wastewater was obtained and PSB was supplied to the set up.

MATERIALS AND METHODS

Collection and culture of photosynthetic bacteria

Wastewater was collected from the wastewater hatchery fishpond at Universiti Putra Malaysia Campus Bintulu (UPMKB) (Refer to Figure 1), with specific coordinates [N 3º12’36.8”; E 113º4’48.3”]. 500 mL of wastewater from each site was used as a starter culture for bacterial cultivation. All samples were transported to the Microbiology and Parasitology Laboratory, UPMKB for the culture process. The microbes grew anaerobically and were exposed to sunlight for approximately 14 days in the sterile bottles containing growth media for the enrichment of purple non-sulfur PSBI colonies until they formed “bloom” (Pfennig, 1967). In the present study, a slight modification was added to the previous technique and named as Cultured PSB (T1).

Water additive experiment with Black Neon Tetra fish

Five treatment groups were designated namely Cultured PSB (T1), positive control (P), negative control (N), and two types of aquatic plant -submerged aquatic plant, hornwort *Ceratophyllum demersum* (T2) and floating aquatic plant, salvinia *Salvinia molesta* (T3). The positive control refers to commercially available PSB while negative control refers to zero treatment solution. The same size glass aquarium square tank (volume 30 cm^3) was used for each treatment. Five individuals of black neon tetra (*H. herbertaxelrodi*) with an average size of (2.5±0.5 cm length) in length were placed in each aquarium and fed with 10 micro pellets of a commercial pellet fish (Tetra, Germany) twice a day every day at 0800 and 1600. The bottom filter (10 cm × 10 cm × 12 cm) was used and packed with biological filters like bio ring media that were mainly used for the PSB to reproduce in the treatment group and positive control group aquarium tank. No biological filter was used at the filter for the aquarium tank in the negative control group. The whole system was set up in triplicate. The fish were reared for 30 days and water parameters such as pH, Ammonia, Nitrate, and Nitrite were recorded.

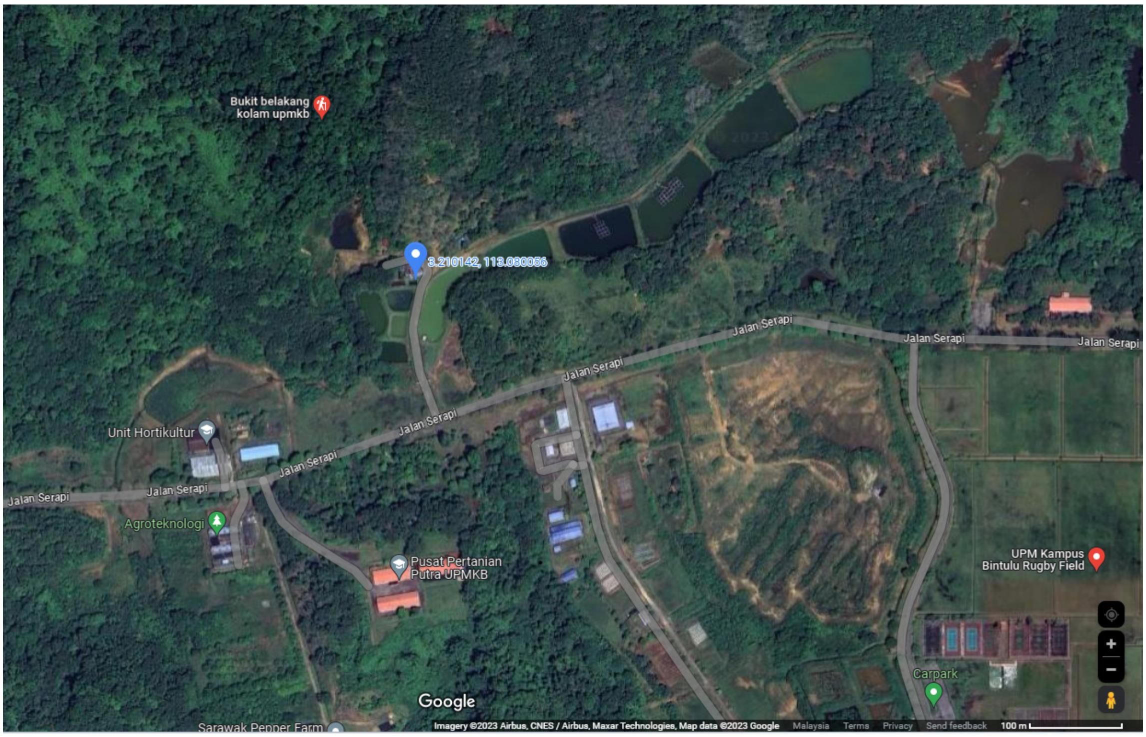

Fig. 1. The sampling site for the photosynthetic bacteria (pointed with blue color) with the exact coordinate 3°12'36.8"N 113°04'48.3"E.

Wastewater treatment

Four treatment groups were designated as aquaculture wastewater from hatchery fishpond (S1), aquaculture wastewater from hatchery fish tank (S2) positive control (P), and negative control (N). The same size glass aquarium square tank (volume 30 cm^3) was used for each treatment. The S1 and S2 groups were supplied with cultured PSB out of total volume, while the positive control refers to commercially available PSB while negative control refers to zero treatment solution of approximately 1% of final volume. Each treatment was prepared in triplicate and equipped with a separate air stone blower (Generic, Air-Stone-Cylinder, India) at room temperature. The experiment was conducted for 30 days and parameters such as pH, ammonia, nitrite, and nitrate were recorded every 3 days interval.

Water quality analysis

Five mL water samples were collected for each aquarium tank. The concentration of ammonia, nitrite, nitrate, and pH levels was measured by using an API freshwater test kit (API Inc., Chalfont, PA, USA). All testing was performed according to the API test kit. To further analyze the UV Spectrophotometer (DLAB Scientific Inc. SP-UV1000, Beijing, China) was performed.

Statistical analysis

All data were recorded in Microsoft Excel 2013. All data were subjected to a one-way analysis of variance (ANOVA). The multiple comparison (Tukey's) test was used to compare the significant differences among treatments using the SPSS computer software (IBM Corp, Armonk, NY, USA). Statistically significant differences required that p values less than 0.05.

RESULTS AND DISCUSSION

Table 1 demonstrates that the concentrations of ammonia, nitrite, and nitrate nitrogen reached their maximum values with the reading of $8.0±0.1^a$, $2.4±0.3^a$, and $41.6±0.8^a$ respectively on day 27 in the negative control group. Based on the obtained results also show that the amount of ammonia and nitrite reached its peak value with $1.0±0.0^{ab}$ and $0.5±0.0^b$ on day 18 of the treatment and started to reduce significantly afterward with the value of $0.3±0.0^c$ ammonia and $0.0±0.0^b$ nitrite at 30th days of the treatment. As for T2, the increasing value was recorded for ammonia and nitrite with $4.0±0.1^b$ and $2.0±0.1^a$ until the last day of the treatment. Meanwhile, there was also a promising pattern was observed in T3 with the reading of ammonia and nitrite starting to decrease 21 days post-treatment. The same pattern was also observed at positive control where the amount of ammonia and nitrite were significantly lower when compared to the negative control. Hence overall it was shown that the concentrations of ammonia and nitrite in T1 and the positive control gradually decreased to amounts below 1.5 ppm by day 30. These results indicate that the application of PSB in T1 and the use of commercial PSB in the positive control effectively improved the water quality of the aquarium tank where black neon tetra fish were reared. It is suggested that PSB in T1 was able to utilize the ammonia and nitrite in the system, converting them into nitrate. Similar findings have shown that PSB can enhance water quality by altering the microbial community structure in the culture systems of Rainbow Trout (Rodríguez Leal *et al*., 2023), Grass Carp (Zhang *et al*., 2014), shrimp (Saejung *et al*., 2021), and few other commercial species.

Puyol *et al*. (2017) also reported that photosynthetic bacteria consume nutrients in wastewater, absorb ammonia, nitrite, and nitrate through photosynthesis, and facilitate biomass recycling for intermediate products. The current study utilized the cultivation of photosynthetic bacteria in wastewater, resulting in significantly lower concentrations of ammonia, nitrite, and nitrate in the wastewater hatchery fishpond (S1) and wastewater from the hatchery fish tank (S2) compared to the negative control group from day 3 until the end of the experiment as displayed in Table 2. It was noted that the amount of ammonia and nitrite started to decrease on Day 12 with 2.0 ± 0.0^{b} and 2.0 ± 0.0^{b} for S1 and 2.0 ± 0.0^{b} and 3.0 ± 0.0^{b} for S2. Based on the obtained value it was strongly suggested that the application of PSB to the wastewater can serve as a supplementary element throughout the culture process which can improve the water quality. This finding aligned with Ying *et al*., (2020) where PSB was able to improve the water quality and enhance the integrity of microbial community composition in integrated multitrophic systems.

Furthermore, the finding from Hülsen *et al*., (2016) also mentioned that the photo-anaerobic treatment in closed systems allows for simultaneous and non-destructive removal of domestic waste. According to Lu *et al*. (2019), this type of wastewater system has the potential to be considered an effective cultivation system for photosynthetic bacteria, enabling simultaneous wastewater treatment and resource recovery. Feasibility studies on PSB wastewater purification have been conducted since 1971 (Lu *et al*., 2018), consistently demonstrating the capability of PSB in wastewater treatment. The macrophyte used in this study, specifically the *S. molesta* plant, demonstrated similar findings to those highlighted by Mustafa and Hayder (2021) regarding its significant role in reducing ammonia and nitrite levels during wastewater remediation. Furthermore, the current study corroborated these results by observing consistently lower nitrogenous compound levels throughout the experiment, attributable to the effective nutrient absorption by *S. molesta* roots, as reported by Anand *et al*. (2017).

CONCLUSION

In conclusion, photosynthetic bacteria (PSB) have the potential to serve as a beneficial supplement for enhancing and maintaining water quality. Additionally, they can be utilized as an alternative treatment method for treating wastewater, particularly within the aquaculture industry.

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ETHICAL STATEMENT

Not Applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1. Isolated photosynthetic bacteria as water additives in maintaining water quality in rearing black neon tetra fish

Treatment	Day	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24	Day 27	Day 30
Isolated PSB (T1)	Ammonia	0.0±0.0[a]	0.1±0.0[b]	0.3±0.0[a]	0.3±0.0[b]	0.5±0.0[b]	0.5±0.0[b]	1.0±0.0[ab]	0.8±0.0[c]	0.4±0.0[c]	0.3±0.0[c]	0.3±0.0[c]
	Nitrite	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[b]	0.3±0.0[a]	0.3±0.0[a]	0.5±0.0[b]	0.4±0.0[b]	0.3±0.0[c]	0.0±0.0[b]	0.0±0.0[b]
	Nitrate	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[b]	0.0±0.0[b]	5.0±0.0[b]	5.0±0.0[b]	10.0±0.0[a]	7.5±0.0[bc]	2.5±0.0 [c]	0.0±0.0[c]	0.0±0.0[c]
	Temperature (°C)	27±0.0[a]	25±0.2[a]	26±0.0[a]	27±0.2[a]	28±0.1[a]	27±0.1[a]	27±0.2[a]	27±0.3[a]	28±0.2[a]	28±0.0[a]	28±0.1[a]
	pH	8±0.0[a]	6±0.2[a]	6±0.1[a]	6±0. [a]	7±0.0[a]	7±0.0[a]	7±0.0[a]	6±0.0[a]	6±0.0[a]	6±0.0[a]	6±0.0[a]
Hornwort Plant (T2)	Ammonia	0.0±0.0[a]	0.1±0.0[b]	0.3±0.0[a]	0.8±0.0[a]	0.5±0.0[b]	1.0±0.0[a]	1.0±0.1[ab]	2.0±0.1[b]	2.0±0.0[b]	4.0±0.0[b]	4.0±0.1[b]
	Nitrite	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.3±0.0[ab]	0.5±0.0[a]	0.5±0.0[a]	0.5±0.0[b]	1.0±0.0[a]	1.0±0.0[b]	2.0±0.0[a]	2.0±0.1[a]
	Nitrate	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[b]	5.0±0.0[ab]	5.0±0.0[b]	5.0±0.0[b]	5.0±0.0[b]	10.0±0.0[b]	10.0±0.0[b]	20.0±0.0[b]	20.6±0.9[b]
	Temperature (°C)	27±0.2[a]	25±0.2[a]	26±0.1[a]	27±0.2[a]	28±0.2[a]	27±0.1[a]	27±0.0[a]	27±0.0[a]	28±0.0[a]	28±0.0[a]	28±0.1[a]
	pH	8±0.0[a]	8±0.1[a]	6±0.1[a]	6±0.1[a]	7±0.0[a]	7±0.0[a]	7±0.0[a]	6±0.0[a]	6±0.0[a]	6±0.0[a]	6±0. [a]
Salvinia Plant (T3)	Ammonia	0.0±0.0[a]	0.0±0.0[b]	0.3±0.0[a]	0.3±0.0[b]	0.5±0.0[b]	0.5±0.0[b]	0.8±0.0[b]	1.0±0.0[c]	0.8±0.0[c]	0.6±0.1[c]	0.5±0.0[c]
	Nitrite	0.0±0.0[a]	0.0±0.0[a]	0.3±0.0[a]	0.3±0.0[ab]	0.3±0.0[a]	0.3±0.0[a]	0.4±0.0[b]	0.5±0.0[b]	0.3±0.0[c]	0.0±0.0[b]	0.0±0.0[b]
	Nitrate	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[b]	0.0±0.0[b]	0.0±0.0[c]	5.4±0.2[b]	5.2±0.4[b]	5.4±0.2[c]	0.3±0.0[c]	0.0±0.0[c]	0.0±0.0[c]
	Temperature (°C)	27±0.0[a]	27±0.0[a]	26±0.0[a]	27±0.2[a]	28±0.2[a]	27±0.0[a]	27±0.1[a]	27±0.1[a]	28±0.0[a]	28±0.1[a]	28±0.1[a]
	pH	8±0.0[a]	8±0.0[a]	6±0.0[a]	6±0.0[a]	7±0.0[a]	7±0.0[a]	7±0.0[a]	6±0.0[a]	6±0.0[a]	6±0.0[a]	6±0.0 [a]
Positive Control (P)	Ammonia	0.0±0.0[a]	0.0±0.0[b]	0.3±0.0[a]	0.3±0.0[b]	0.5±0.0[b]	0.5±0.0[b]	1.0±0.1[ab]	0.8±0.0[c]	0.4±0.0[c]	0.3±0.0[c]	0.3±0.0[c]
	Nitrite	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[b]	0.3±0.0[a]	0.3±0.0[a]	0.5±0.0[b]	0.4±0.0[b]	0.3±0.0[c]	0.0±0.0[b]	0.0±0.0[b]
	Nitrate	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[b]	0.0±0.0[b]	5.0±0.0[b]	5.0±0.0[b]	7.5±0.1[ab]	7.5±0.0[bc]	0.3±0.0[c]	0.0±0.0[c]	0.0±0.0[c]
	Temperature (°C)	27±0.0[a]	25±0.0[a]	26±0.0[a]	27±0.2[a]	28±0.2[a]	27±0.0[a]	27±0.0[a]	27±0.0[a]	28±0.3[a]	28±0.1[a]	28±0.1[a]
	pH	8±0.1[a]	6±0.0[a]	6±0.0[a]	6±0.0[a]	7±0.0[a]	7±0.0[a]	7±0.0[a]	6.4±0.1[a]	6±0.0[a]	6±0.0[a]	6±0.0[a]
Negative Control (N)	Ammonia	0.0±0.0[a]	0.5±0.0[a]	0.5±0.0[a]	1.0±0.0[a]	1.0±0.0[a]	1.1±0.1[a]	1.5±0.1[a]	3.0±0.1[a]	4.0±0.1[a]	8.0±0.1[a]	8.0±0.1[a]
	Nitrite	0.0±0.0[a]	0.0±0.0[a]	0.3±0.0[a]	0.5±0.0[a]	0.5±0.0[a]	0.5±0.0[a]	1.0±0.0[a]	1.1±0.1[a]	2.0±0.1[a]	2.4±0.3[a]	2.0±0.1[a]
	Nitrate	0.0±0.0[a]	0.3±0.0[a]	5.0±0.0[a]	7.5±0.0[a]	10.0±0.0[a]	10.0±0.3[a]	10.2±0.5[a]	20.0±0.7[a]	20.4±0.7[a]	41.6±0.8[a]	40.2±1.0[a]
	Temperature (°C)	27±0.0[a]	25±0.0[a]	26±0.0[a]	27±0.2[a]	28±0.2[a]	27±0.2[a]	27±0.1[a]	27±0.2[a]	28±0.0[a]	28±0.2[a]	28±0.1[a]
	pH	8±0.0[a]	6±0.0[a]	6±0.0[a]	6±0.0[a]	7±0.0[a]	7±0.0[a]	7±0.0[a]	7±0.2[a]	6±0.0[a]	6±0.0[a]	6±0.0[a]

Table 2. Wastewater treatment analysis by using isolated photosynthetic bacteria

Wastewater	Day	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	Day 24	Day 27	Day 30
Hatchery fishpond (S1)	Ammonia	8.0±0.0^{a}	4.0±0.6^{a}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{a}
	Nitrite	6.0±0.0^{a}	6.0±0.0^{a}	3.0±0.0^{b}	3.0±0.0^{b}	2.0±0.0^{b}	1.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
	Nitrate	160.0±0.0^{a}	160.0±0.0^{a}	80.0±0.0^{b}	20.0±0.0^{b}	20.0±0.0^{b}	10.0±0.0^{b}	5.0±0.6^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
	Temperature	28.0±0.0^{a}	28.0±0.0^{a}	28.0±0.3^{a}	27.0±0.3^{a}	27.0±0.6^{a}	27.0±0.0^{a}	27.0±0.6^{a}	28.0±0.5^{a}	27.0±0.6^{a}	27.0±0.6^{a}	27.0±0.6^{a}
	pH	6.6±0.0^{a}	6.6±0.1^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.1^{a}	6.4±0.1^{a}	6.5±0.1^{a}	6.4±0.1^{a}	6.4±0.0^{a}	6.5±0.1^{a}	6.4±0.1^{a}
Hatchery fish tank (S2)	Ammonia	8.0±0.0^{a}	4.0±0.6^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{a}
	Nitrite	6.3±0.9^{a}	6.0±0.0^{a}	6.0±0.0^{ab}	5.0±0.0^{ab}	3.0±0.0^{b}	2.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
	Nitrate	160.0±0.0^{a}	160.0±0.0^{a}	80.0±0.0^{b}	20.0±0.0^{b}	20.0±0.0^{b}	20.0±0.0^{b}	10.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
	Temperature	28.0±0.0^{a}	27.7±0.3^{a}	28.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}	28.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}
	pH	6.6±0.0^{a}	6.6±0.1^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}
Positive Control (P)	Ammonia	8.0±0.0^{a}	4.0±0.6^{b}	4.0±0.0^{ab}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{a}
	Nitrite	6.0±0.0^{a}	6.0±0.0^{a}	4.0±0.0^{b}	4.0±0.0^{b}	2.0±0.0^{b}	2.0±0.0^{b}	1.0±0.0^{b}	1.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
	Nitrate	160.0±0.0^{a}	160.0±0.0^{a}	160.0±0.0^{a}	40.0±0.0^{b}	40.0±0.0^{b}	20.0±0.0^{b}	10.0±1.0^{b}	5.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
	Temperature	28.0±0.0^{a}	28.0±0.0^{a}	28.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}	28.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}
	pH	6.6±0.0^{a}	6.6±0.0^{a}	6.6±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}	6.4±0.0^{a}
Negative Control (N)	Ammonia	8.0±0.0^{a}	7.7±0.3^{a}	7.7±0.3^{a}	7.7±0.3^{a}	8.3±0.3^{a}	8.3±0.3^{a}	8.3±0.3^{a}	9.0±1.0^{a}	9.3±0.7^{a}	9.3±0.7^{a}	10.0±1.0^{a}
	Nitrite	8.0±0.0^{a}	8.0±0.0^{a}	8.0±0.0^{a}	7.3±0.6^{a}	7.0±0.0^{a}	7.0±0.0^{a}	7.0±0.0^{a}	8.0±0.0^{a}	8.0±0.0^{a}	8.0±0.0^{a}	8.0±0.0^{a}
	Nitrate	160.0±0.0^{a}	160.0±0.0^{a}	160.0±0.0^{a}	160±0.0^{a}	160.0±0.0^{a}	160.0±0.0^{a}	160.0±0.0^{a}	160.0±0.0^{a}	160.0±0.0^{a}	160.0±0.0^{a}	160.0±0.0^{a}
	Temperature	28.0±0.0^{a}	28.0±0.0^{a}	28.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}	27.0±0.0^{a}	27.0±0.6^{a}	28.0±0.0^{a}	27.0±0.6^{a}	27.0±0.6^{a}	27.0±0.6^{a}
	pH	6.6±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}

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