Heliyon 10 (2024) e24996

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Analysis of changes in nutrient salts and other water quality indexes in the pond water for largemouth bass (micropterus salmoides) farming

Junyi Qiu [a,1], Chunyan Zhang [a,1], Zhaojun Lv [a], Zhen Zhang [a], Yuxuan Chu [a],
Dongwei Shang [b], Yibo Chen [b], Chengxun Chen [a,*]

[a] *Tianjin Key Laboratory of Aquatic Ecology and Aquaculture, College of Fisheries, Tianjin Agricultural University School, Tianjin, 300384, China*
[b] *Tianjin Jiahe Tianyuan Ornamental Fish Culture Co., Ltd., Tianjin, 301823, China*

ARTICLE INFO

Keywords:
Largemouth bass
Freshwater pond
Experimental data
Trend change
Nutrient salts
Water quality index

ABSTRACT

To explore the changes in nitrite nitrogen, ammoniacal nitrogen, nitrate nitrogen, phosphates, pH, dissolved oxygen, salinity, and water temperature over time and the correlations and mutual influences between these indexes in the traditional farming of largemouth bass, this study selected three ponds in Lizigu Farm in Baodi District of Tianjin, China as research objects. From May to October 2021, nutrient salts and other water quality indexes in the ponds were measured, and water samples were collected at different depths for repetition, Water is collected from the ponds using Plexiglas samplers and sent back to the lab for determination of water quality indexes using our national laboratory standards. According to the analysis of the measurement results, in traditional farming, nitrite nitrogen, ammoniacal nitrogen, nitrate nitrogen, phosphates, pH, dissolved oxygen, salinity, and water temperature in the ponds for largemouth bass all change significantly over time, with different changing trends and certain correlations with each other. In particular, nutrient salts indexes in ponds are influenced by other water quality indexes, human activities, and phytoplankton. During the breeding process, strengthening the dynamic monitoring of nutrient salts and other water quality indexes in the ponds and adjusting the nitrogen, phosphorus, and ammonia levels in the ponds artificially play an important role in preventing eutrophication in the water and promoting the green and sustainable production of pond ecosystems, in particular, allowing better quality growth of the largemouth bass, as well as ensuring the production and economic efficiency. This study provides a theoretical basis and data support for further optimization of traditional pond aquaculture in similar regions, in order to provide aquatic products with better quality and achieve higher economic benefits.

1. Introduction

Aquatic products, an important part of food in modern agriculture, serve as one of the important material foundations, so the farming methods of aquatic products naturally become a key topic of discussion. Pond farming, as the most important and widely used

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[*] Corresponding author.
E-mail address: ccxnxy@163.com (C. Chen).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e24996
Received 19 April 2023; Received in revised form 7 January 2024; Accepted 18 January 2024

method, occupies a significant position in global aquaculture, especially in China. Traditional pond aquaculture plays a pivotal role in the development of freshwater aquaculture, and pond aquaculture accounts for a large proportion in all farming methods. Then largemouth bass, as one of the larger fish species in pond culture, has been studied by many researchers on its pond culture conditions, especially in terms of pond water quality. Similar to most carnivorous fish, the largemouth bass prefers to survive in clear water with the temperatures of about 20 °C–30 °C, a suitable pH of 6–8.5 and a water dissolved oxygen requirement of 4 mg/L or more. This kind of fish has a wide range of salinity adaptations, it can live not only in fresh water but also in salty and brackish water with a salinity of up to 10 ‰. When the nitrite and ammonia levels are high, the feeding of it can be affected. The fish selected for this trial were also largemouth bass, in order to make the findings more generalisable.

However, in many parts of the world where traditional pond aquaculture is carried out, farmers lack systematic monitoring, statistics, and summaries of changes in water quality throughout the aquaculture process. Therefore, they cannot precisely improve and optimize water quality during the aquaculture process or accurately evaluate the dynamic changes in pond water, which may adversely affect economic benefits, for example, in the summer of 2020, in the area where this trial was conducted, there was an increase in mortality of bass due to high nitrite nitrogen in the ponds, which was not detected and the actions were not taken in time, resulting in economic losses. Good water quality is a prerequisite for the entire culture process in traditional ponds, and maintaining a balanced level of water quality indexes is the basis for the healthy growth of cultured fish, which is a crucial condition for ensuring fish productivity. Therefore, it is recommended to regularly monitor and evaluate pond water quality to promote healthier and more sustainable fish culture.

In recent years, an increasing number of studies on the impact of water quality on aquaculture have been conducted by researchers around the world. For example, Indian researchers have experimented and found that the levels of soluble inorganic nitrogen and soluble inorganic phosphorus can significantly change the primary productivity of aquaculture ponds. Their findings indicated that a deficiency in nitrogen and phosphorus can limit the production of autotrophic biomass in aquaculture ponds, consequently affecting the ecological balance of fish ponds [1]. Chinese researchers, by varying the quantity of rice cultivation within the same system, demonstrated the ability to regulate the nutrient cycling in aquaculture ponds. They found that compared to single-fish farming, the co-cultivation of rice significantly reduced the concentration of NH4+ in the ponds, thereby promoting fish growth [2]. Croatian researchers studied the effect of vertical mixing between deep and surface water on the nutrient status of fish pondsand found that water mixing led to the downward transport of dissolved oxygen and the upward influx of nutrients into the surface layer, thus promoting ammonium flow and facilitating nitrification in fish ponds [3]. Portuguese researchers adjusted the water quality of aquaculture ponds by introducing seagrass into the water source reservoir. They discovered that the presence of seagrass maintained adequate oxygen levels in the fish ponds and effectively controlled pathogens [4]. Pakistani researchers ensured a smooth aquaculture process by regularly monitoring and evaluating the water quality in the aquaculture ponds. They concluded that good water quality is fundamental to the healthy growth of fish, especially during the breeding season [5]. Almost all of the current researches show the apparent importance of maintaining good water quality for farmed fish.

This study conducted a five-month (153 days in total) monitoring and periodic sampling of pond water quality in Tianjin, China, where traditional pond farming methods are used. The fish farmed in the ponds was largemouth bass. The dynamic changes of nutrient salts, such as nitrite nitrogen (Nitrites), nitrate nitrogen (Nitrates), ammoniacal nitrogen (AN), and phosphates (PHOS), and other water quality indexes, such as pH, dissolved oxygen (DO), salinity (SAL), and water temperature (WT) in the culture water were regularly monitored to explore the relationships between various indexes and reveal the changes in the pond water during the entire culture process, which can provide a meaningful reference for areas where similar traditional pond aquaculture is widely used, facilitating practices such as regulating the water environment, promoting sustainable aquaculture, and preventing eutrophication in the water , in particular, allowing better quality growth of largemouth bass, as well as ensuring the production and economic efficiency.

2. Materials and methods

2.1. Experimental conditions

The fish ponds used in this experiment were provided by Tianjin Jiahe Tianyuan Ornamental Fish Culture Co. Ltd. Three ponds were selected for periodic sampling. Pond 1 covers an area of 5086.7 m^2, with a depth of 2.0 m; Pond 2 covers an area of 4826.7 m^2, with a depth of 2.3 m; Pond 3 covers an area of 4786.7 m^2, with a depth of 2.3 m. The fish were released on May 10, 2021, with 7540 in Pond 1, 9330 in Pond 2, and 11,960 in Pond 3. The initial weight of the fish was (54.00 ± 2.30) g, and the sizes were similar. After 153 days, the fish were caught and sold on October 8, 2021. Each pond was equipped with three aerators, with an engine power of 3kw. During the culture period of largemouth bass, the water temperature of the ponds ranged between 20.75 °C and 29.25 °C. Before the official outset of this trail, we carried out some index measurements on the source of the pond water, that is, on the water body of the impounding reservoir. The method for the determination of PH is the glass electrode method (GB 6920-86), for nitrite is the diazo coupling method (HJ 168–2010), for ammonia nitrogen is the nano reagent method (HJ 535–2009) and for dissolved oxygen is the iodometric method (GB 7489-87), the determination methods for the chemical components in the above parentheses are all derived from: '*The National Standards of the People's Republic of China*' jointly issued by the State Administration for Market Regulation and the Standardization Administration of the People's Republic of China, the measured pH was 8.28 (mg/L), nitrite nitrogen was 0.02 (mg/L), ammonia nitrogen was 0.02 (mg/L) and dissolved oxygen was 8.19 (mg/L).

2.2. Content of the experiment

Three ponds were selected and sampled regularly for five months, 153 days in total. The interval between every two samples was 15 days. The changing trends of Nitrites, Nitrates, AN, PHOS, PH, DO, SAL, and WT at each stage were investigated, and the correlations between nutrient salts and other water quality indexes in pond water under traditional culture conditions were also examined. The dates of sampling were 9 May (Z1), 30 May (Z2), 15 June (Z3), 30 June (Z4), 15 July (Z5), 30 July (Z6), 15 August (Z7), 30 August (Z8), 15 September (Z9), and 30 September (Z10).

The aerators were turned on every sunny morning for two to 3 h and in the second half of the night on cloudy days. In addition, the aerators were turned on to maintain dissolved oxygen above 4 mg/L when the pond water lacked oxygen.

Within a week after sampling, once all experimental results have been determined, decisions regarding the necessity of water exchange and other supplementary measures are made based on the experimental outcomes, other supplementary measures include chlorine dioxide and citric acid. Chlorine dioxide, a potent oxidizing and disinfecting agent, is applied quantitatively to the ponds during one morning from 9 a.m. to 10 a.m. within the week after sampling. It is evenly distributed throughout the entire pond at a concentration of 0.1 mg/L to 0.2 mg/L. Citric acid, it is mainly used to provide carbon sources for ponds. The main approach to water exchange was pumping out some of the pond water and adding some new water. Before water, one third of the water in the ponds was pumped out. A large rubber skin hose was used to pump water from the main reservoir, and the water inlet was filtered with a dense mesh to prevent debris from entering the fish pond.

2.3. Experimental methods

During each sampling of the pond water, the researcher rowed a boat to the central point of the small ponds to collect water samples from the upper and lower layers. Two organic glass water samplers with a volume of 4 L were used for each sample collection. One sampler was used for upper-layer water sampling (50 cm below the water surface), and the other sampler for lower-layer water sampling (50 cm above the sediment layer). After sampling one pond, the researcher quickly poured the collected water into six white polyethylene plastic bottles with a capacity of 500 mL, with three bottles for the upper layer water and three bottles for the lower layer water. Therefore, all the indexes of each sampling had six measured values. All water samples were placed in an insulated box with ice packs for low-temperature and light-shielded storage before being brought back to the laboratory for further analysis.

The remaining water in the glass water samplers was poured into another two white polyethylene plastic bottles with a capacity of 500 mL for the observation of phytoplankton. Specifically, 1 % Lugol's solution was added immediately to the water sample for fixation. The supernatant was removed using a siphon tube after the mixture stood for 48 h and was adjusted to a volume of 40–50 mL for microscopic observation of the phytoplankton.

The methods for measuring nutrient salts and water quality indexes are as follows: the diazotization coupling colorimetric method (GB7493-87) for nitrite nitrogen, the vanillin assay method (GB/T6682-2008) for nitrate nitrogen, the Nessler's reagent method (HJ535-2009) for ammoniacal nitrogen, the molybdenum blue method (GB12763.4–91) for phosphates, the glass electrode method (GB6920-86) for pH, the iodine method (GB7489-87) for dissolved oxygen, the determination methods for the chemical components in the above parentheses are all derived from: '*The National Standards of the People's Republic of China*' jointly issued by the State Administration for Market Regulation and the Standardization Administration of the People's Republic of China, and a HANNA electronic salinity meter (equipment model: HI98319) for salinity, since the portable dissolved oxygen meter is very accurate for measuring water temperature, the water temperature was also measured using the meter (equipment model: JPBJ-608) purchased from Shanghai INESA (Group) Co., Ltd. The microscope used to observe the phytoplankton was Olympus (equipment model: SZ61TR).

2.4. Data statistics and analysis

Data processing and statistical analysis were carried out using Excel 2019 and SPSS 22.0 software, respectively. One-way ANOVA was conducted, and Turkey posthoc test was used for multiple comparisons ($P < 0.05$). The results were presented as mean ± standard

Table 1
Changes in nitrite nitrogen in different ponds.

project	Pond I	Pond II	Pond III
Z1	0.19 ± 0.01^{f}	0.16 ± 0.01^{g}	0.14 ± 0.01^{f}
Z2	0.20 ± 0.02^{ef}	0.20 ± 0.01^{f}	0.14 ± 0.01^{f}
Z3	0.22 ± 0.02^{e}	0.23 ± 0.01^{e}	0.13 ± 0.02^{f}
Z4	0.31 ± 0.01^{d}	0.20 ± 0.01^{f}	0.20 ± 0.00^{e}
Z5	0.41 ± 0.01^{c}	0.23 ± 0.02^{e}	0.91 ± 0.02^{a}
Z6	0.88 ± 0.02^{a}	0.92 ± 0.02^{b}	0.91 ± 0.01^{a}
Z7	0.88 ± 0.03^{a}	0.95 ± 0.01^{a}	0.93 ± 0.02^{a}
Z8	0.58 ± 0.02^{b}	0.33 ± 0.02^{d}	0.27 ± 0.02^{d}
Z9	0.57 ± 0.02^{b}	0.34 ± 0.01^{d}	0.61 ± 0.01^{b}
Z10	0.29 ± 0.02^{d}	0.87 ± 0.01^{c}	0.53 ± 0.02^{c}

Note: Different letters in the superscripts of figures in the same row indicate a significant difference ($P < 0.05$); the same letter or no letter indicates an insignificant difference ($P > 0.05$). The same applies to the subsequent tables.

deviation. Origin 21.0 software was used for Pearson correlation analysis of related data and graph drawing.

3. Results

3.1. Changes in nutrient salts

3.1.1. Changes in nitrite nitrogen

Table 1 shows that nitrite nitrogen concentrations in Pond 2 and Pond 3 exhibited an overall increasing-decreasing-increasing trend, while the concentration in Pond 1 showed an increasing-decreasing trend. The nitrite nitrogen concentrations in all three ponds peaked during the period from Z5 to Z7 and gradually declined between Z7 and Z8. The concentration in Pond 1 continued to decrease between Z8 and Z10, and the concentration in Pond 2 gradually rebounded during the same period. In Pond 3, the concentration recovered between Z8 and Z9 before dropping again between Z9 and Z10.

3.1.2. Changes in nitrate nitrogen

Table 2 shows that the nitrate nitrogen concentrations in all three ponds exhibited an overall fluctuating trend during the period from Z1 to Z6, followed by a continuous upward trend between Z6 and Z7. The nitrate nitrogen concentrations in all three ponds peaked at Z7, decreased between Z7 and Z8, and showed an upward trend again between Z9 and Z10.

3.1.3. Changes in ammoniacal nitrogen

Table 3 shows that ammoniacal nitrogen concentrations in all three ponds exhibited a slight downward trend between Z1 and Z2, an upward trend between Z2 and Z3, a decrease between Z3 and Z4, and a rising trend between Z4 and Z5. The ammoniacal nitrogen concentrations in Pond 1 and Pond 2 both peaked at Z5, after which the concentrations in Pond 1 and Pond 3 decreased between Z5 and Z8 and rebounded between Z8 and Z9 before dropping again between Z9 and Z10. In Pond 3, the ammoniacal nitrogen concentration exhibited a decrease between Z5 and Z7 before continuously rising between Z7 and Z10.

3.1.4. Changes in phosphates

Table 4 shows that phosphate concentrations in all three ponds exhibited an overall fluctuating trend between Z1 and Z5, followed by a continuous increase between Z5 and Z6. The concentrations in all three ponds peaked at Z6. After that, the figures in Pond 1 and Pond 3 continuously decreased between Z6 and Z8 and rebounded between Z8 and Z10, while the concentration in Pond 2 continuously dropped between Z6 and Z9 before rising between Z9 and Z10.

3.2. Changes in other water quality indexes

3.2.1. Changes in pH

As shown in Table 5, pH values in all three ponds continued to drop between Z1 to Z2 and exhibited fluctuating trends between Z2 to Z6. Afterward, the pH values in all three ponds increased between Z6 to Z8 and then dropped again between Z8 and Z9. The difference was that pH values in Pond 1 and Pond 3 began to rise between Z9 and Z10 while the value in Pond 2 continued to drop during this period.

3.2.2. Changes in dissolved oxygen

As shown in Table 6, dissolved oxygen in Pond 1 showed an overall fluctuating trend without a significant increase or decrease. Although similar trends were observed in the other ponds, they exhibited more noticeable fluctuations. Specifically, dissolved oxygen in Pond 2 increased between Z1 and Z3, dropped between Z3 and Z4, increased again between Z4 and Z6, and continuously decreased between Z6 and Z10. Dissolved oxygen in Pond 3 decreased between Z1 and Z2, rose between Z2 and Z3, dropped again between Z3 and Z4, and sharply increased between Z4 and Z6, followed by an insignificant change between Z6 and Z7, a decrease between Z7 and Z8, and another increase between Z8 and Z10.

Table 2
Changes in nitrate nitrogen in different ponds.

project	Pond I	Pond II	Pond III
Z1	0.015 ± 0.004^{de}	0.012 ± 0.001^{f}	0.018 ± 0.002^{d}
Z2	0.024 ± 0.004^{d}	0.034 ± 0.002^{c}	0.075 ± 0.002^{c}
Z3	0.015 ± 0.004^{e}	0.015 ± 0.003^{f}	0.011 ± 0.001^{e}
Z4	0.027 ± 0.006^{cd}	0.013 ± 0.002^{f}	0.016 ± 0.002^{de}
Z5	0.014 ± 0.005^{e}	0.011 ± 0.001^{f}	0.012 ± 0.001^{e}
Z6	0.035 ± 0.006^{c}	0.015 ± 0.003^{f}	0.016 ± 0.002^{de}
Z7	0.071 ± 0.008^{a}	0.079 ± 0.003^{a}	0.085 ± 0.004^{a}
Z8	0.015 ± 0.004^{e}	0.021 ± 0.002^{e}	0.011 ± 0.001^{e}
Z9	0.009 ± 0.002^{e}	0.026 ± 0.001^{d}	0.016 ± 0.003^{de}
Z10	0.056 ± 0.004^{b}	0.075 ± 0.002^{b}	0.056 ± 0.004^{b}

Table 3
Changes in ammoniacal nitrogen in different ponds.

project	Pond I	Pond II	Pond III
Z1	0.27 ± 0.03^{h}	0.25 ± 0.03^{h}	0.26 ± 0.02^{g}
Z2	0.24 ± 0.02^{h}	0.24 ± 0.03^{h}	0.32 ± 0.04^{g}
Z3	2.23 ± 0.07^{d}	2.42 ± 0.10^{c}	2.40 ± 0.10^{c}
Z4	1.40 ± 0.06^{f}	1.71 ± 0.04^{e}	2.10 ± 0.08^{d}
Z5	4.34 ± 0.08^{a}	5.30 ± 0.07^{a}	3.18 ± 0.08^{b}
Z6	2.64 ± 0.07^{c}	1.91 ± 0.09^{d}	1.48 ± 0.06^{e}
Z7	2.09 ± 0.02^{e}	1.16 ± 0.06^{g}	1.23 ± 0.03^{f}
Z8	1.25 ± 0.04^{g}	1.49 ± 0.07^{f}	1.46 ± 0.03^{e}
Z9	3.78 ± 0.07^{b}	2.35 ± 0.08^{c}	3.66 ± 0.03^{a}
Z10	3.77 ± 0.07^{b}	3.73 ± 0.05^{b}	3.74 ± 0.06^{a}

Table 4
Changes in phosphates in different ponds.

project	Pond I	Pond II	Pond III
Z1	0.09 ± 0.02^{e}	0.12 ± 0.02^{g}	0.28 ± 0.04^{g}
Z2	0.16 ± 0.03^{e}	0.32 ± 0.03^{f}	0.39 ± 0.04^{f}
Z3	0.19 ± 0.02^{de}	0.83 ± 0.04^{c}	0.80 ± 0.05^{c}
Z4	0.26 ± 0.05^{d}	0.75 ± 0.04^{c}	0.79 ± 0.04^{c}
Z5	0.17 ± 0.04^{e}	0.37 ± 0.07^{f}	0.25 ± 0.03^{g}
Z6	3.27 ± 0.07^{a}	3.46 ± 0.04^{a}	3.55 ± 0.09^{a}
Z7	2.36 ± 0.04^{b}	1.74 ± 0.07^{b}	2.67 ± 0.05^{b}
Z8	0.37 ± 0.06^{c}	0.66 ± 0.06^{d}	0.39 ± 0.05^{f}
Z9	0.33 ± 0.04^{cd}	0.46 ± 0.03^{e}	0.54 ± 0.04^{e}
Z10	0.32 ± 0.04^{cd}	0.83 ± 0.03^{c}	0.65 ± 0.04^{d}

Table 5
Changes in pH in different ponds.

project	Pond I	Pond II	Pond III
Z1	9.17 ± 0.03^{a}	9.36 ± 0.08^{a}	8.95 ± 0.07^{a}
Z2	8.34 ± 0.09^{c}	8.35 ± 0.06^{c}	8.37 ± 0.10^{cd}
Z3	8.28 ± 0.05^{c}	8.36 ± 0.08^{c}	8.31 ± 0.07^{cd}
Z4	8.35 ± 0.14^{c}	8.35 ± 0.08^{c}	8.30 ± 0.01^{d}
Z5	8.42 ± 0.08^{bc}	8.34 ± 0.10^{c}	8.25 ± 0.03^{d}
Z6	8.37 ± 0.05^{c}	8.28 ± 0.05^{c}	8.29 ± 0.04^{d}
Z7	8.42 ± 0.02^{bc}	8.37 ± 0.12^{bc}	8.58 ± 0.05^{b}
Z8	8.54 ± 0.04^{b}	8.50 ± 0.03^{b}	8.65 ± 0.03^{b}
Z9	8.39 ± 0.09^{c}	8.30 ± 0.03^{c}	8.30 ± 0.03^{d}
Z10	8.43 ± 0.07^{bc}	8.25 ± 0.03^{c}	8.40 ± 0.04^{c}

Table 6
Changes in dissolved oxygen in different ponds.

project	Pond I	Pond II	Pond III
Z1	8.08 ± 0.25^{a}	8.28 ± 0.77^{bc}	10.93 ± 0.19^{a}
Z2	7.08 ± 0.13^{ab}	8.83 ± 0.50^{b}	8.05 ± 1.10^{bc}
Z3	7.34 ± 0.15^{ab}	11.96 ± 1.10^{a}	8.32 ± 0.83^{b}
Z4	6.82 ± 0.90^{ab}	6.93 ± 0.83^{c}	8.03 ± 0.11^{bc}
Z5	6.35 ± 0.96^{b}	8.34 ± 0.26^{bc}	9.26 ± 0.85^{b}
Z6	7.36 ± 0.94^{ab}	10.43 ± 0.38^{ab}	10.39 ± 0.57^{ab}
Z7	6.97 ± 1.40^{ab}	9.38 ± 0.14^{b}	10.05 ± 0.89^{ab}
Z8	7.10 ± 1.25^{ab}	6.35 ± 1.33^{c}	6.15 ± 0.90^{c}
Z9	5.83 ± 0.40^{b}	6.12 ± 1.02^{c}	6.18 ± 0.92^{c}
Z10	6.14 ± 0.44^{b}	5.50 ± 1.00^{c}	7.02 ± 0.83^{c}

3.2.3. Changes in salinity

As shown in Table 7, salinity in Pond 1 exhibited a fluctuating trend between Z1 and Z4, increased between Z4 and Z6, decreased between Z6 and Z8, and then rose again between Z8 and Z10. Salinity in Pond 2 decreased between Z1 and Z2, increased between Z2 and Z3, and continuously declined between Z3 and Z8 before rising again between Z8 and Z10. Salinity in Pond 3 continued to increase between Z1 and Z4, decreased between Z41 and Z6, rebounded between Z6 and Z7, dropped again between Z7 and Z9, and picked up between Z9 and Z10.

3.2.4. *Changes in water temperature*

As shown in Table 8, due to seasonal changes, the water temperatures in all three ponds first rose and then dropped.

3.3. *Correlations between nutrient salts and other water quality indexes*

The correlations between nutrient salts and other water quality indexes were also examined. The results may not be universally applicable due to the limited number of ponds and sampling points, but they can still provide a reference for the full-cycle aquaculture in most ponds. Fig. 1 shows that there is a significant positive correlation between nitrite nitrogen and phosphates in Pond 1, and Figs. 2 and 3 show that there is also a positive correlation between nitrite nitrogen and phosphates in Ponds 2 and 3. In addition, there is a significant negative correlation between ammoniacal nitrogen and dissolved oxygen in Pond 1, and Figs. 2 and 3 suggest a negative correlation between ammoniacal nitrogen and dissolved oxygen in Ponds 2 and 3. There is a significant positive correlation between nitrite nitrogen and water temperature in Pond 1, and similarly, there is also a positive correlation between nitrite nitrogen and water temperature in the other two ponds.

Fig. 2 shows that there is a significant positive correlation between nitrite nitrogen and nitrate nitrogen in Pond 2, and Figs. 1 and 3 show that there is also a positive correlation between nitrite nitrogen and nitrate nitrogen in the other two ponds. In addition, there is a significant positive correlation between nitrite nitrogen and phosphates in Pond 2 and a positive correlation between nitrite nitrogen and phosphates in Pond 1 and Pond 3.

Fig. 3 shows that there is a significant positive correlation between nitrite nitrogen and water temperature in Pond 3, and similarly, there is a positive correlation between nitrite nitrogen and water temperature in the other two ponds.

In addition, the correlations between other factors are shown in the heatmaps below and will not be further elaborated.

3.4. *Phytoplankton in the ponds*

To better explain the changing trends of nutrient salts and other water quality indexes, the changes in algae in the culture ponds during the farming process were also explored. In Fig. 4, from May to October 2021, 196 species of phytoplankton were identified in 10 sampling surveys of the culture water in the ponds for largemouth bass, including 5 variants, belonging to 6 phyla and 55 genera. The phylum with the most species was green alga, with 19 genera and 90 species, followed by diatom, with 15 genera and 35 species, blue-green alga, with 10 genera and 34 species, euglena, with 5 genera and 20 species, dinoflagellate, with 4 genera and 5 species, and Cryptophyta, with 2 genera and 3 species.

4. Analysis and discussion

4.1. *Analysis of the changes in nutrient salts*

If the concentration of nitrite nitrogen is high, it will mainly lead to the conversion of haemoglobin in the fish body into high iron haemoglobin, causing the haemoglobin to gradually lose the ability to convey oxygen and causing hypoxia in the fish, which, if not monitored and detected in time, may even result in the death of the largemouth bass, further causing irreversible losses to the production. Thus, the trial monitored the changes in nitrite and nitrate nitrogen throughout the culture process and analysed their causes, allowing them to be improved quickly and systematically, targeting bass production to save some of the economic losses. From the perspective of traditional pond aquaculture, the main reasons for the increase in concentrations of nitrite nitrogen and nitrate nitrogen in the three ponds between Z5 and Z7 are the continuous increase in the stocking density, improper feeding methods, long-term lack of pond sediment dredging, slowing down of the reproduction rate of beneficial algae in the ponds, and seasonal changes in water temperature. In terms of the fish stocking density in a pond, as the fish grow, the stocking density continues to increase, which directly leads to a gradual oxygen deficit in the pond. As a result, nitrogen-containing organic matter begins to decompose, ultimately producing ammoniacal nitrogen and nitrite nitrogen [6], which will be further oxidized into nitrate nitrogen [7]. In terms of improper

Table 7
Changes in salinity in different ponds.

project	Pond I	Pond II	Pond III
Z1	2.50 ± 0.14^{ab}	2.24 ± 0.06^{bc}	1.12 ± 0.26^{b}
Z2	1.74 ± 0.63^{b}	1.32 ± 0.01^{c}	2.74 ± 0.16^{ab}
Z3	2.33 ± 0.35^{ab}	3.17 ± 0.41^{a}	3.23 ± 0.54^{a}
Z4	2.00 ± 0.02^{b}	2.76 ± 0.26^{ab}	3.23 ± 0.54^{a}
Z5	2.13 ± 0.08^{b}	2.42 ± 0.30^{b}	2.74 ± 0.68^{ab}
Z6	3.17 ± 0.41^{a}	2.17 ± 0.41^{bc}	2.09 ± 0.20^{b}
Z7	1.75 ± 0.63^{b}	1.66 ± 0.42^{c}	2.25 ± 0.81^{ab}
Z8	1.64 ± 0.67^{b}	1.60 ± 0.23^{c}	2.02 ± 0.81^{b}
Z9	1.75 ± 0.48^{b}	1.77 ± 0.62^{bc}	1.52 ± 0.76^{b}
Z10	1.94 ± 0.52^{b}	1.79 ± 0.61^{bc}	1.72 ± 0.04^{b}

Table 8
Changes in water temperatures in different ponds.

project	Pond I	Pond II	Pond III
Z1	20.57 ± 0.41^{h}	20.07 ± 0.08^{g}	20.45 ± 0.10^{h}
Z2	23.73 ± 0.44^{f}	22.47 ± 0.15^{e}	22.57 ± 0.23^{f}
Z3	25.37 ± 0.15^{d}	25.47 ± 0.12^{c}	25.12 ± 0.20^{d}
Z4	25.55 ± 0.14^{d}	25.70 ± 0.19^{bc}	25.63 ± 0.23^{cd}
Z5	28.70 ± 0.09^{ab}	28.88 ± 0.52^{a}	28.77 ± 0.53^{ab}
Z6	29.12 ± 0.13^{a}	29.17 ± 0.15^{a}	29.25 ± 0.24^{a}
Z7	28.28 ± 0.51^{b}	28.48 ± 0.60^{a}	28.30 ± 0.52^{b}
Z8	26.30 ± 0.51^{c}	26.20 ± 0.52^{b}	26.03 ± 0.23^{c}
Z9	24.38 ± 0.13^{c}	24.63 ± 0.57^{d}	24.40 ± 0.19^{e}
Z10	21.47 ± 0.18^{g}	21.67 ± 0.26^{f}	21.53 ± 0.19^{g}

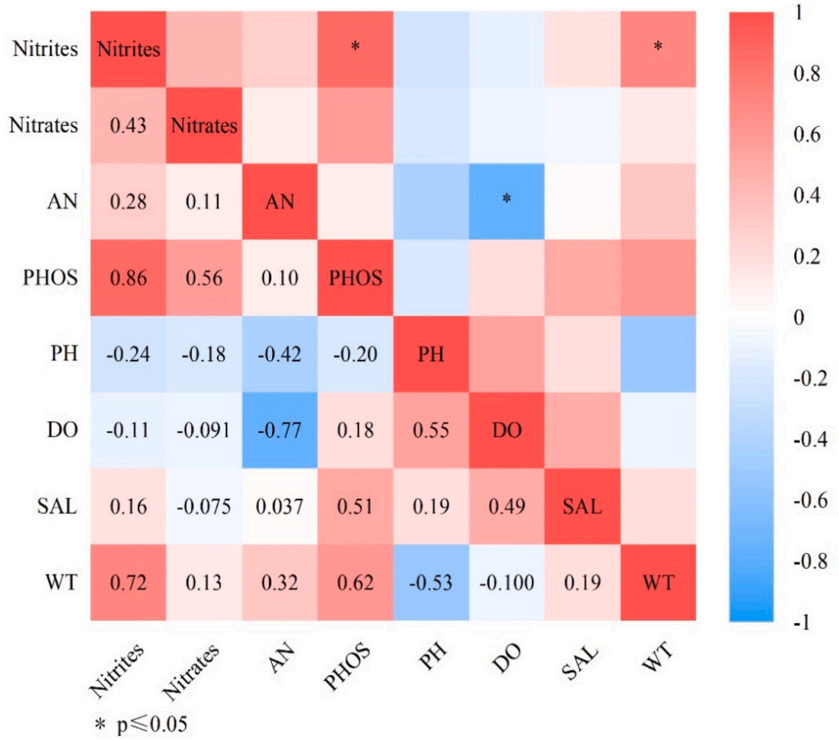

Fig. 1. Heatmap of correlations between nutrient salts and other water quality indexes in Pond 1.

feeding methods, farmers often feed fish excessively, and some of the feed is discharged into the water before being digested by the fish. During the decomposition of excrement and residual feed, a large amount of ammonia and toxic substances are produced [8], which will then be transformed into nitrite nitrogen and nitrate nitrogen by phototrophic bacteria and nitrite bacteria [9]. The slowing down of the reproduction rate of beneficial algae in a pond can also lead to the generation of nitrite nitrogen. Nitrite nitrogen and ammoniacal nitrogen in a pond can be absorbed in large quantities through a series of chemical reactions when there are sufficient beneficial algae in the pond [10]. However, a large amount of ammoniacal nitrogen and nitrite nitrogen cannot be absorbed when beneficial algae are not sufficient. Similarly, nitrate nitrogen cannot be timely absorbed by algae as nutrient salts and may be reduced to nitrite nitrogen by denitrifying bacteria [11]. This series of reactions could increase the nitrite nitrogen in pond water. Moreover, the growth of beneficial and harmful algae in the pond is also a crucial factor. For instance, green alga, diatom, and Cryptophyta can efficiently utilize ammonia. Therefore, when these kinds of algae become the dominant species, the nutrient content in the pond would show a favorable trend. Conversely, when harmful algae such as blue-green alga, dinoflagellate, and Euglena grow vigorously, the ammonia content in the pond will start to increase due to their poor capability of utilizing ammonia. Furthermore, the long-term accumulation of harmful algae has a negative impact on the conversion of substances and the healthy growth of fish in the pond. In addition, if the pond sediment is not cleaned for a long time, substances at the bottom of the pond such as sludge will decompose and ferment, consuming oxygen during decomposition [12] and producing nitrite nitrogen during fermentation [13]. Also, water temperature is a reason for the varying concentrations of nitrite nitrogen and nitrate nitrogen in the three ponds. As illustrated in the figures and tables, the activity of nitrifying bacteria and denitrifying bacteria in the ponds increased between Z5 and Z7 with higher

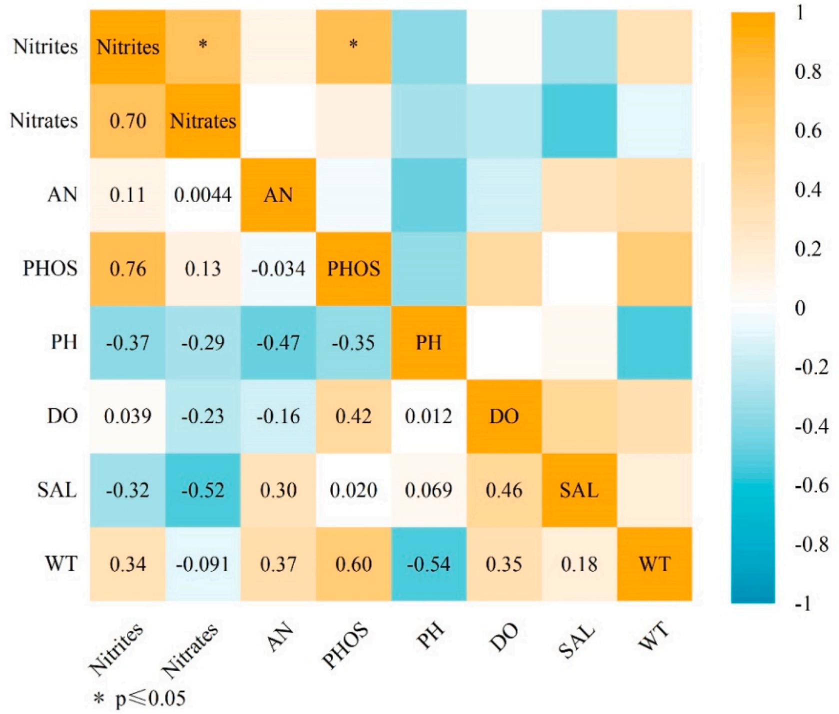

Fig. 2. Heatmap of correlations between nutrient salts and other water quality indexes in Pond 2.

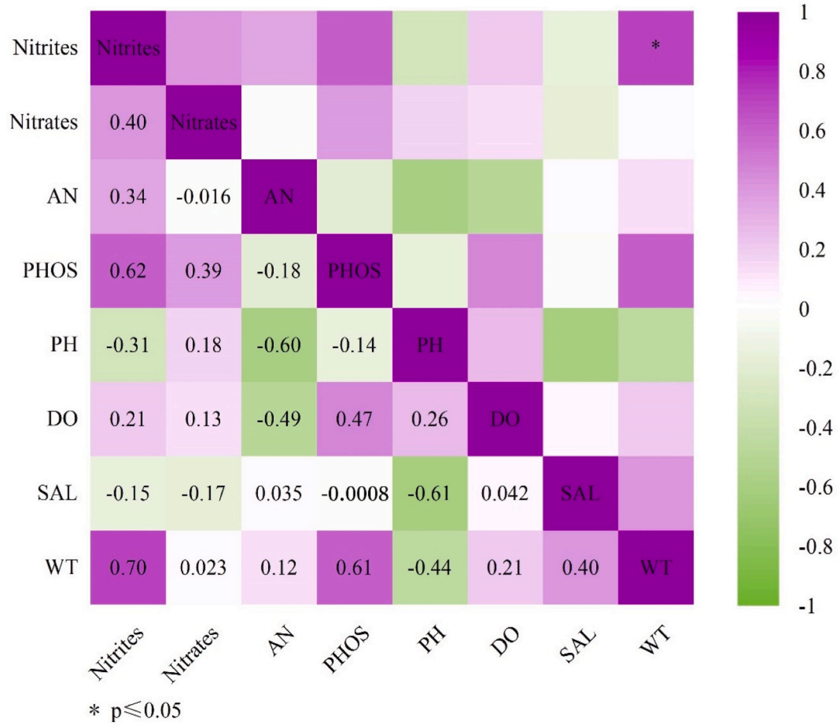

Fig. 3. Heatmap of correlations between nutrient salts and other water quality indexes in Pond 3.

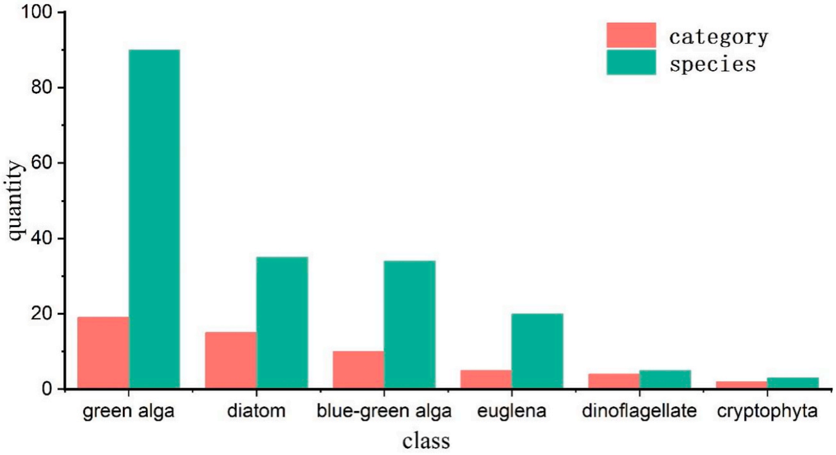

Fig. 4. Biomass of phytoplankton in culture ponds.

temperatures [14]. This resulted in the production of nitrate nitrogen, which was easily reduced to nitrite nitrogen, hence the concentration of nitrite nitrogen rose during this period. In summary, all of the reasons mentioned above lead to an increase in the concentrations of nitrite nitrogen and nitrate nitrogen in pond water.

The following analysis reveals the causes of the rise in ammoniacal nitrogen throughout the culture process, which is also a very important indicator in the culture process. Although the largemouth bass is a highly ammonia-tolerant fish, high levels of ammonia may still bring about oxidative stress, inflammatory responses and reduced immunity in the body of the bass, even leading to mortality. This trial therefore monitored the changes in ammonia nitrogen throughout the culture process and analysed the causes, which could be improved in time to save the loss of bass production. The main reasons for the increased ammoniacal nitrogen concentrations between Z3 and Z5 in all three ponds were inappropriate feeding, low content of nitrifying bacteria in the ponds, insufficient dissolved oxygen, and increased rainfall. Firstly, overfeeding leads to waste and excess residual feed in ponds, resulting in the spoilage of a large amount of high-protein feed. Nitrogen-containing organic matter is decomposed by bacteria to produce ammonia, which affects the carbon-nitrogen ratio in the pond and breaks the balance [15], driving up the ammoniacal nitrogen concentration. Secondly, when the content of nitrifying bacteria in the pond is low (i.e., insufficient nitrite and nitrate bacteria), the nitrification process cannot be smoothly completed [16], resulting in an accumulation of ammoniacal nitrogen. Thirdly, ammoniacal nitrogen in the pond can be converted to nitrite nitrogen and nitrate nitrogen with sufficient oxygen, while the conversion cannot proceed smoothly with insufficient dissolved oxygen [17], resulting in more accumulation of ammoniacal nitrogen. The fourth reason is excessive rainfall caused by weather changes between Z3 and Z5. Due to the increased rainfall, some of the pollutants accumulated in the soil were discharged into the ponds with surface runoff, and the sediment at the bottom of the ponds further accumulated, thus leading to an increase in the ammoniacal nitrogen concentration [18]. Moreover, if the ammoniacal nitrogen concentration remains high, other reasons, in addition to the above ones, maybe a shortage of beneficial algae that can utilize ammonia and low oxidation rates of ammonia. Additionally, the release of ammonia from fish increases under high temperatures.

During the breeding period, the concentrations of nitrite nitrogen, nitrate nitrogen, and ammoniacal nitrogen in the ponds intermittently decreased because the farmers took some measures. However, due to differences in the effectiveness and intensity of the solutions, the changing trends of nitrite nitrogen, nitrate nitrogen, and ammoniacal nitrogen in the three ponds differed to some extent.

Overall, the levels of nitrite nitrogen, ammoniacal nitrogen, and nitrate nitrogen can indirectly reflect the production intensity in the ponds , It is the changes in these indexes that affects the growth and production of our object studied throughout the culture process. At the same time, their changing trends under dynamic conditions also reflect the level of ammonia utilization and conversion. The root cause of high nitrite concentration is the obstruction of ammonia oxidation. Furthermore, during the entire breeding process, there may be a decrease in ammonia and nitrates and an increase in nitrites, which may be due to some other factors other than oxygen and bacteria. The former may be utilized by beneficial algae, while the latter is rejected by beneficial algae. Further exploration and in-depth research are necessary for regulating and managing the entire breeding process.

While phosphates are not directly toxic to fish, high phosphate levels can lead to excessive algal growth in water. Phosphates have a more significant effect on algal growth than nitrates, which can indirectly affect fish growth. Additionally, phosphates are acidic and can lower the alkalinity of pond water. Therefore, measures need to be taken to control and regulate the phosphate concentrations in ponds.

The main reasons for the increased phosphate concentrations in all three ponds were insufficient water exchange during the breeding period [19], overfeeding leading to the production of excess feed waste and fish excrement, which increased the phosphate levels in the water [20], failure to regularly clean the sediment at the bottom of the ponds at the end of each breeding cycle, which could cause an increase in phosphate levels in the pond water as the sediment decomposed [21], possible phosphorus pollution of water sources [22], and a lack of caution of farmers in selecting water treatment chemicals as some of these chemicals used to change water quality may contain phosphate components [23]. The last reason is less likely to have an impact.

4.2. Analysis of the changes in other water quality indexes

The reasons for changes in PH throughout the culture process, which is also a very important index in the process. When the pH of the pond water is too high, it principally causes a reduction in the fish's desire to eat, which in turn has some influences on its growth. The trial therefore monitored the changes in PH throughout the culture process and analysed the causes, allowing rapid and planned improvements to be made against PH deviation, enabling the largemouth bass to grow more healthily. The main reasons for the high pH levels at the early stage were seasonal changes, temperature changes, and the use of some artificial microecologics. Due to the season, the temperatures in the ponds were a bit low at the beginning of the culture period, which was one of the reasons for the high pH values. As the temperature rises, acidification may occur when the water becomes warm, and the pH value gradually decreases [24]. At the initial stage of the breeding period, the farmers added artificial microecologics similar to probiotics to regulate the water quality, but the pH levels were high due to the inappropriate amount of microecologics used [25]. The pH values in the ponds began to drop between Z1 and Z2 because of the release of largemouth bass and other species for polyculture that increased the culture density, and a large amount of carbon dioxide was released through respiration, which pulled down the pH values [26]. When the bottom sediment has not been cleaned for a long time, it can be overly acidic, which, along with the decaying organic matter, causes a gradual decrease in the pH value [27]. Another reason might be weak photosynthesis due to insufficient phytoplankton, which will also lead to a decrease in the pH value in the water [28]. In addition, part of the reason why pH values in the ponds gradually leveled off in the later stages was that the number of phytoplankton species was regulated to a relatively appropriate level. Researchers have explored the growth of phytoplankton in their ecosystem and its correlation with pH value and found aquaculture water environments can be optimized by improving the ecological regulation ability of phytoplankton [29]. The result that the phytoplankton abundance in ponds is correlated with pH value can provide a theoretical basis for the building of ecological aquaculture ponds with appropriate pH levels.

Dissolved oxygen is a critically important factor in aquaculture, and only sufficient dissolved oxygen can maximize the economic benefits of aquaculture animals，It is an important milestone to ensure the final production of the bass. During the culture process, dissolved oxygen levels in the water may decrease sometimes despite sufficient aerators probably because the rate of oxygen consumption exceeds the rate of supplementation and organic substances increase and accumulate without being regularly cleaned. These substances consume a large amount of oxygen during decomposition [30]. Excessively high contents of nutrient elements such as nitrogen and phosphorus can lead to eutrophication in the water [31], resulting in an imbalance in the distribution of phytoplankton, an increase in phytoplankton abundance, a decrease in water transparency, and an increase in oxygen consumption by plant respiration [32]. All these factors gradually reduce the concentration of dissolved oxygen. Moreover, during feeding periods, largemouth bass need oxygen when eating [33], consuming dissolved oxygen in the ponds.

The reason for exploring salinity throughout the culture process is that when the salinity of the pond is too high, the osmotic pressure in the largemouth bass will gradually rise, directly resulting in a disequilibrium in osmoregulation and ion regulation in the fish, making the physiological condition of the fish not functioning properly and leading to an increase in the mortality of the fish in the pond, so this trial also monitors the changes in salinity throughout the culture process and analyses the causes, allowing rapid and planned improvements in salinity to save some of the economic losses in terms of bass production. Precipitation, evaporation, and solubility are the main factors affecting the fluctuations in salinity in the ponds. Increased rainfall causes a decrease in salinity [34]. As mentioned above, the period from Z3 to Z5 saw a lot of rainfall, and salinity in the ponds declined during this period. Under normal circumstances, in the summer with higher temperatures, salinity increases as a larger amount of water is evaporated and the solubility of salt also rises [35]. However, in this study, salinity in the ponds did not increase. The reason was a large amount of water replacement due to the poor states of most water quality indexes (nitrite nitrogen, nitrate nitrogen, and ammoniacal nitrogen were all overly high). Because of the freshwater sources, salinity did not significantly rebound [36]. Towards the end of the breeding period, salinity in the ponds gradually stabilized and increased slightly. This may be due to the dry autumn with plentiful sunshine and a certain degree of evaporation, which increases salinity [37]. In addition, the number of phytoplankton species in the ponds was regulated to a relatively appropriate level in this stage. Researchers have found salinity of water can be improved by improving the ecological regulation ability of phytoplankton and the number of phytoplankton species is correlated with salinity [38].

Last, water temperatures in all three ponds generally exhibited an upward trend followed by a downward trend primarily due to seasonal changes. During spring and summer, water temperatures increase, followed by a decline starting in autumn [39,40].

4.3. Analysis of the correlations between nutrient salts and other water quality indexes

As the maintenance of good water quality is of particular importance for the growth of largemouth bass, the correlation between these water quality indexes will also be analysed below, so that the water quality can be systematically regulated through the implication and control of each index, giving subtle improvements for the water quality in the pond.

Fig. 1 shows the correlations between nutrient salts and other water quality indexes in Pond 1. Specifically, there is a significant positive correlation between nitrite nitrogen and phosphates, a significant negative correlation between ammoniacal nitrogen and dissolved oxygen, and a significant positive correlation between nitrite nitrogen and water temperature. The correlations between the above indexes are the same in the other two ponds.

Fig. 2 shows the correlations between nutrient salts and other water quality indexes in Pond 2. Specifically, there is a significant positive correlation between nitrite nitrogen and nitrate nitrogen and a significant positive correlation between nitrite nitrogen and phosphates. The correlations between the above indexes are the same in the other two ponds.

Fig. 3 shows the correlations between nutrient salts and other water quality indexes in Pond 3. Specifically, there is a significant positive correlation between nitrite nitrogen and water temperature. The correlation between the above indexes is the same in the other two ponds.

The experimental results show that there is a negative correlation between ammoniacal nitrogen concentration and dissolved oxygen concentration probably because high water temperatures during the breeding period accelerate the respiration rate of living organisms, leading to an insufficiency of dissolved oxygen in the water and thus causing dissimilatory nitrate reduction that produces ammonia. During this process, nitrates are converted to the more toxic ammoniacal nitrogen [41]. The organic matter accumulated also increases the concentration of ammoniacal nitrogen in the water through anaerobic decomposition [42]. Therefore, a decrease in dissolved oxygen results in an increase in ammoniacal nitrogen in the ponds. The reasons for the positive correlation between the nitrite nitrogen concentration and water temperature in the ponds are the feeding of largemouth bass and vigorous metabolic activities. Meanwhile, as a large amount of feed is placed in the water and the metabolic products of organisms are dissolved in the water, the higher water temperature promotes microbial activity in the water and sediment [43]. As a result, organic substances are more intensely decomposed, with nitrite nitrogen released [44]. According to the experimental results, the changing trends of the nitrite concentration and nitrate concentration were largely the same. As mentioned above, there are many common causes of increased nitrite nitrogen and nitrate nitrogen, such as improper feeding, sediment that has not been clean for a long time, and changes in water temperature in different seasons. The main reason is the accumulation of organic matter in the ponds during the culture process and the long-term anaerobic environment, which leads to the anaerobic decomposition of microorganisms and drives up the concentrations of nitrite and nitrate nitrogen in the ponds. Another reason might be that the changes in nitrite nitrogen and nitrate nitrogen depend on the changes in ammoniacal nitrogen [45]. Studies have found that ammoniacal nitrogen is the main inorganic compound decomposed from the excreta of organisms and is also the primary product of inorganic nitrogen from the decomposition of carcasses [46]. Researchers have demonstrated that higher levels of ammoniacal nitrogen and a highly reducing environment in culture water can be detrimental to the oxidation of nitrogen [47].

5. Conclusions

Throughout the breeding period, various indexes in the ponds for largemouth bass, including nitrite nitrogen, ammoniacal nitrogen, nitrate nitrogen, phosphates, pH, dissolved oxygen, and salinity, changed significantly over time, with different changing trends, and there were certain correlations between nutrient salts and other water quality indexes , and the water quality can be systematically regulated through the implication and control of individual indexes. Based on the fluctuations in these water quality indicators, it can be inferred that adjustments are needed in the cultivation practices for largemouth bass. Of particular significance is the observation that at certain stages of cultivation, the trends in nutrient salt changes suggest that nitrogen and phosphorus are not undergoing a beneficial cycle, leading to the accumulation of some harmful metabolites. This indicates a lack of appropriate nitrifying and denitrifying bacterial strains in the pond, preventing beneficial algae from reproducing normally and affecting the aquatic environment for largemouth bass. Furthermore, aside from the shortage of suitable bacterial strains, the changing trends in pond nutrient salts are partly influenced by the excrement of largemouth bass. To address this situation, it is essential to investigate and evaluate the carbon-to-nitrogen ratio in feed ingredients and the presence of phosphates in preservatives. Therefore, the water quality of a pond has a crucial impact on the growth and productivity of largemouth bass. It is imperative to regularly monitor nutrient salts and other water quality parameters and take timely measures to address fluctuations in aquaculture pond water quality based on their interrelationships.

Author Statement

Junyi Qiu、Chunyan Zhang：Conduct of the experiment, sample collection, data measurement and analysis, writing of the first draft. Zhaojun Lv：Sample collection ,measure biochemical parameters. Zhen Zhang：Determination of biochemical indicators , operation of scientific instruments. Dongwei Shang 、Yibo Chen：Provision of test sites, funding, writing reviews. Yuxuan Chu：Sample collection, operation of scientific instruments. Chengxun Chen：Experimental design, funding, writing reviews and revisions. All authors have reviewed the manuscript.

Data availability statements

The data generated in this study are included in the article and any further reasonable requests can be made by contacting the appropriate author.

Ethical statement

This experiment did not use any animals or humans as experimental consumables.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare no competing interests.SMARTQC

Acknowledgement

The present study obtained Tianjin Science and Technology Plan Project (21ZYCGSN00020) , Tianjin Freshwater Aquaculture Industry Technology System Innovation Team Construction Project (ITTFRS2021000) and Tianjin Baodi District Agricultural Science and Technology Plan Project (202005) .

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