POLYCULTURE AS AN EFFECTIVE APPROACH FOR THE LONG-TERM MANAGEMENT OF WATER ENVIRONMENT AND FISH HEALTH: A CASE STUDY OF A SOCIAL-CULTURAL HERITAGE FISH POND

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ABSTRACT

Managing a culturally and historically important fish pond poses unique challenges compared to conventional fish ponds. This study explores the role of fish population structure in the water environment and fish health in Ho Chi Minh Fish Pond, an intensive and valuable historical fish pond located in the Presidential Palace in Hanoi, Vietnam. Seventeen aquaculture species, categorized into six groups based on their habitat within distinct water layers and their position in the food chain, were carefully selected to optimize energy utilization in the pond. Water quality parameters (temperature, dissolved oxygen, pH, $P\text{-}PO_4$, $N\text{-}NO_2$, $N\text{-}NH_4$, H_2S, chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total suspended solids (TSS), *Aeromonas*, and *Streptococcus* spp. counts, zooplankton, and phytoplankton were monitored from 2018 to 2022. In addition, fish health was assessed by pathogen infection rate (parasites, bacteria, and viruses), and the fish condition factor (CF). The research findings reveal that a combination of a common carp dominant group (67.68% in mass) with plankton filter group (9.03%), plant diet group (1.23%), omnivorous plant-diet based group (13.55%), and predator group (5.76%) maintained a stable ecological condition and good fish health condition with CF from 0.92-2.41. The water environment had seasonal variations, with increased organic pollution and pathogen proliferation during the summer months. *Aeromonas* and *Streptococcus* spp. counts in water positively correlated to zooplankton density (R= 0.50 and 0.68, respectively), emphasizing the need for effective zooplankton density control. This study highlights the importance of fish population structure for maintaining the ecological balance and fish health.

Keywords: *Fish conservation, polyculture, landscape, environment, fish pathogen.*

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1. INTRODUCTION

Aquaculture, with its long historical development, holds significant implications for global food security. Numerous aquaculture ponds around the world bear invaluable cultural and societal significance, contributing to the rich tapestry of cultural heritage. Preserving these aquaculture ponds becomes of utmost importance. However, the conservation of these aquaculture models demands a profound understanding of aquaculture practices and ecosystems. For this purpose, ponds are subject to a variety of management approaches aimed at sustainable and biodiversity conservation. Conservation initiatives involve a combination of strategies impacting various organism groups directly and indirectly [1]. A significant challenge in conservation biology is the formulation of effective management programs that sustain and enhance biodiversity in human-altered landscapes [2, 3]. In the realm of pond complex conservation management, there is a predominant focus on fish stock management. The manipulation of fish stocks can exert a profound influence on fish communities and, in turn, affect other organism groups directly through predation [4] or indirectly through alterations in the pond environment including, nutrient cycling, physical disturbance [5, 6]. The drainage regime is another critical factor with direct impacts on multiple organism groups, including fish, and may also induce important indirect effects by modifying pond environmental conditions [7, 8]. Management interventions such as sediment removal can change the pond environment by reducing nutrient loading [9].

Polyculture in aquaculture has a relatively long history of development [10]. Polyculture promotes species compatibility and sustainability, enhancing productivity, resource sharing, environmental protection, and animal welfare. However, each model has unique attributes [11]. In polyculture, only a proper combination of ecologically different species at adequate densities can utilize the available resources efficiently, and maximize the synergistic fish-fish and fish-environment relationships [12]. In other words, optimal fish population structuring helps maximize nutrient utilization in the pond, minimize the risk of pollution, and maintain ecological balance. Effective polyculture requires managing species compatibility to avoid predation and competition, ensuring well-being and overall system productivity. However, understanding ecological functions and managing potential interspecies pathogen spillover when designing species combinations and stocking densities is essential. Combining certain species may trigger pathogen spillover [13, 14]. To mitigate this risk, it is advisable to use indigenous species in polyculture systems rather than introducing exotic ones. Studies [15, 16] reported that the seabass-tilapia polyculture system effectively addressed breeding issues while supporting sustainable production. Wang *et al*. (2022) [17] reported that polyculture, which includes yellow catfish, grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), and bighead carp (*Hypophthalmichthys nobilis*), provided better production and environmental conditions. This system

resulted in higher oxygen levels and lower levels of ammonia nitrogen, nitrite nitrogen, total suspended solids, chemical oxygen demand, nitrate nitrogen, orthophosphate, and chlorophyll a (Chl-a), compared to monoculture (catfish) and biculture (yellow catfish and grass carp).

In freshwater ponds, environmental parameters, including temperature, pH, dissolved oxygen (DO), hydrogen sulfide (H_2S), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), ammonia nitrogen ($N\text{-}NH_4$), total suspended solids (TSS), and plankton, serve as crucial indicators for water quality and diversity assessment [1]. Concurrently, disease pathogens in water, such as *Aeromonas* spp. and *Streptococcus* spp., are common risks. Additionally, in the context of freshwater aquaculture, diseases caused by parasites, bacteria, and viruses, such as Koi herpes virus (KHV) and Spring viremia of carp (SVC), pose common and significant risks [18, 19]. The interplay between these ecological indicators and disease risks underscores the intricate dynamics that shape the health and biodiversity of pond ecosystems, necessitating comprehensive and science-based management strategies [18, 19]. Akazawa *et al*. (2014) [20] suggested that environment and disease surveillance programs can help to minimize the risk of disease in aquaculture.

Ho Chi Minh Fish Pond at the Presidential Palace is a socio-cultural heritage site in Hanoi, Vietnam. According to President Ho Chi Minh's Vestige in the Presidential Palace area, in 2019 (before the COVID-19 period), the Presidential Palace received 2.5 million domestic and foreign visitors. Preserving the landscape and conserving the fish population in the ponds are of significant importance.

This study assesses the effect of fish population structure on long-term changes in water quality, fish disease pathogens, and fish health in Ho Chi Minh Fish Pond. By examining the long-term implications of environment monitoring, disease surveillance, and fish population health, the research aims to shed light on the dynamic interplay between aquaculture practices, environmental conditions, disease prevalence, and the overall well-being of the fish population. This comprehensive analysis contributes valuable insights for sustainable and effective aquaculture management strategies.

2. MATERIALS AND METHODS

2.1. Study area

This research was carried out at Ho Chi Minh Fish Pond at the Ho Chi Minh Presidential Palace, Ba Dinh ward, Hanoi, Vietnam from January 2018 to December 2022. The fish pond covers an area of 3,320 square meters with an average depth of 2.36 meters and is surrounded by a large garden. The pond houses 16 species of fish, including 11 species that were raised by President Ho Chi Minh during his stay here, one approximately 30 kilogram turtle, and five added species (for optimizing the population structure). According to Giang (2018) [21], the estimated total fish weight in the pond was approximately 4,700 kilograms, equivalent to 1.43 kilograms per square meter. Additionally, the fish pond has unique characteristics as a historical site, which poses certain challenges for its

management. The pond's banks are covered with many ancient trees, which regularly shed leaves and create debris, affecting the fish's living environment. The fish pond is located in the city center, with a shortage of clean water supply. Meanwhile, the installation of auxiliary facilities to enhance water filtration and aeration is restricted due to their impact on the overall landscape and the historical significance of the heritage site. In addition, climate change has also posed a risk to aquaculture [22]. Extreme weather events such as heavy rain and extreme temperature variations may significantly affect the water quality of the pond. It is recorded that heavy rain flushes out the wastewater from the garden, which enriches nutrients and decreases the oxygen level in Ho Chi Minh Fish Pond [21]. These issues present many challenges for fish pond management. Inappropriate environmental management can impact the surrounding environment (poor color and odor) and even jeopardize the safety of the fish population.

2.2. Management of environment, fish health, and fish population

Since 1978, the Research Institute for Aquaculture No. 1 has overseen President Ho Chi Minh's pond, preserving its historical fish population. Major renovations in 2013 restructured the population, maintaining 11 original species and adding 5 new species. Between 2013 and 2018, minor adjustments were made to the fish population composition to facilitate management and conservation. From 2018 to 2022, the fish population structure has stabilized, contributing to improved water quality and preventing disease risks. Fish population structure was maintained by balancing the documented fish deaths, eliminations, and the new introduction of fish. The principle for managing the population is to preserve the historical integrity of 17 species and to optimize the structure by adjusting the number of each species. Renovations occur every 5-8 years, with accurate assessments made only during these events. In October 2021, significant renovations, including sediment removal and fish reassessment, were conducted. The primary management process, detailed in Supplementary Material (SM) 1, includes environmental, disease, and fish population management.

2.2.1. Water environmental monitoring and analysis

Water temperature, pH, and dissolved oxygen (DO) were monitored by an automatic water temperature/pH/DO monitoring system (AWMS) TWT Model DIQ/S284. Data on pH, DO, and temperature were measured and reported every 15 minutes. $N\text{-}NH_4$, $N\text{-}NO_2$, H_2S, chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), $P\text{-}PO_4$, total suspended solids (TSS), phytoplankton, zooplankton**,** *Aeromonas* spp., and *Streptococcus* spp. in the water were sampled once a month. In case of a disease outbreak or an abnormal phenomenon, fish and water were additionally sampled. On average, five additional sampling events were conducted per year. Water samples were collected from three points during each sampling event to represent the general water quality.

Water was sampled by a Wildco bathometer (S/N:1120-G42) at a depth of 30 cm. Samples were stored at 3-5^{o}C and transferred within 3 hours to an ISO

17025:2017 accredited laboratory for analysis. Hydrochemical variables were analyzed following Baird and Bridgewater (2017) [23]. N-NO_2, N-NH_4, P-PO_4, COD, and H_2S were determined by colorimetric method using Hitachi U-2900 spectrophotometer (S/N:30E13-007) following the instruction in SMEWW 4500-$NO2^-$ B:2017, SMEWW 4500-NH3 B,F:1017, SMEWW 4500-P B,E:2017, SMEWW 5200 C:2017, SMEWW 4500-$S2^-$ B,D:2017, respectively. TSS was quantified by calculating the weight after drying at 103-105^oC following instructions in SMEWW 2540 D:2017. BOD_5 was measured after 5 days of incubation at 20°C following the instructions in ISO5815 1,2:2003. Zooplankton and phytoplankton were identified to species or genera where possible under a compound and stereoscopic microscope (Olympus CX23 S/N: RTFS2, StemDV4) following instructions in SMEWW 1200 B:2017. Plankton individual density was determined by Sedgwick-Rafter counting cell (S/N:3-1801-G20).

Streptococcus spp. and total *Aeromonas* spp. counts were quantified according to Buller (2004) [24]. All bacterial colonies were enumerated, and the results are presented as colony-forming units/mL (CFU/mL). The details of the bacteria identification methods are presented in SM2.

2.2.2. Fish disease surveillance and analysis

In total, 125 common carp and 125 tilapia individuals were collected within 5 years. During each sampling event, 3-10 fish were collected; 5 sampling events were carried out each year. Abnormal swimming behavior fish or fish with signs of disease were sampled using a dip net. Fish were transported in live condition with an oxygen supply to the laboratory.

Identification of bacteria in fish: After transferring the fish to the laboratory, their livers and kidneys were dissected and isolated on nutrient agar. The nutrient agar plates, once inoculated, were incubated in a culture cabinet at 29 °C for 24 hours. Subsequently, the isolated bacteria were subcultured onto nutrient agar plates and incubated at 29 °C for another 24 hours. The isolated bacteria were then identified by being harvested and subjected to biochemical testing using the API 20E kit. The identification results were read using the apiweb™ software: https://apiweb.biomerieux.com/login (bioMérieux, France).

Identification of parasites in fish: Parasite examination on the skin and gills was performed according to Te and Ky (2007) [25] by collecting mucus from the skin and gills, making fresh smears, and observing under a microscope (at 4X, 10X, and 40X magnification). The infection level of parasites was characterized by the infection rate (IR) and calculated as follows: IR (%) = (Number of infected samples /Total number of examined samples) x 100.

Identification of virus in fish: Koi herpesvirus (KHV) in carp was detected by PCR following the method described by Gray *et al*. (2002) [26]. Liver and gill tissues were homogenized, and DNA was extracted using GeneAll® Exgene TM Kit (GeneAll, Korea) following the manufacturer's instructions and used for analysis. Details of the virus identification method are presented in SM2.

2.3. Statistical analysis

- Condition factor (CF) was calculated as CF = (weight (g)/length^3 (cm)) × 100.

- Differences in the average values of the monitored water parameters between months and years were analyzed using SPSS statistical software (version 23, IBM Corp). First, the data were tested for normality using the Shapiro-Wilk test. If the normality condition was satisfied, ANOVA was used to determine whether there were any significant differences between the groups. A nonparametric Kruskal-Wallis test was used if the normality condition was not satisfied. A significant difference was recognized when $p < 0.05$.

- Principal Component Analysis was performed by Statistica 12 (StatSoft). The mean values of 14 parameters from hydro-physicochemical, phytoplankton, and zooplankton variables were normalized using z-score values before analysis.

- Pearson correlation was tested to find the relationship between all investigated parameters (hydrophysicochemical, zooplankton, and phytoplankton variables). Data from all years were polled.

3. RESULTS AND DISCUSSION

3.1. Water quality variation

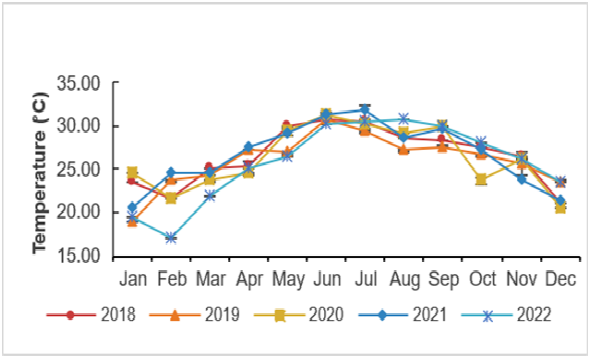
The variations of water parameters in the Ho Chi Minh Fish Pond from 2018 to 2022 are presented in table 1.

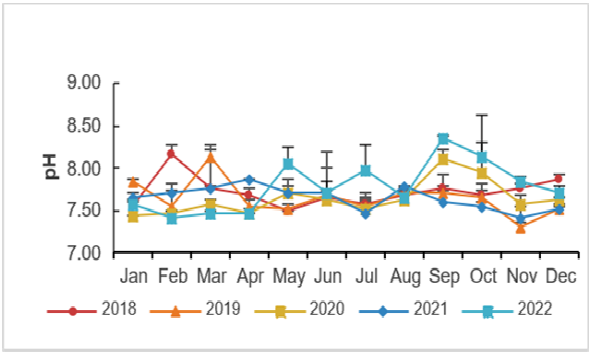
Table 1. Yearly variation of water parameters in Ho Chi Minh Fish Pond from 2018-2022

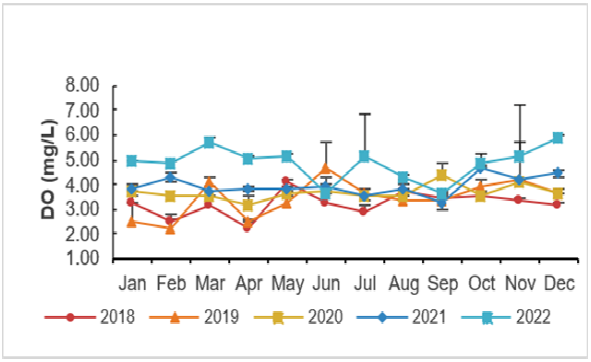
Years	2018	2019	2020	2021	2022
Water temperature (^oC)	26.58 ± 3.27^a	26.06 ± 3.11^a	26.23 ± 3.67^a	26.71 ± 3.72^a	25.79 ± 4.55^a
pH	7.72 ± 0.18$^{a\,b}$	7.65 ± 0.21^a	7.64 ± 0.21^a	7.64 ± 0.14^a	7.85 ± 0.37^b
DO (mg/L)	3.21 ± 0.52^a	3.47 ± 0.75^a	3.67 ± 0.31^a	3.67 ± 0.4^a	5.33 ± 1.33^b
N-NH_4 (mg/L)	0.34 ± 0.29^a	0.25 ± 0.20^a	0.25 ± 0.13^a	0.46 ± 0.20^a	0.47 ± 0.26^a
P-PO_4 (mg/L)	0.015 ± 0.014^a	0.014 ± 0.016^a	0.028 ± 0.019^a	0.019 ± 0.013^a	0.023 ± 0.022^a
N-NO_2 (mg/L)	0.32 ± 0.17^a	0.26 ± 0.13^a	0.35 ± 0.26^a	0.39 ± 0.28^a	0.35 ± 0.24^a
H_2S (mg/L)	0.015 ± 0.010^b	0.019 ± 0.005^b	0.004 ± 0.004^a	0.005 ± 0.009^a	0.003 ± 0.003^a
COD (mg/L)	17.11 ± 4.69^c	11.39 ± 4.86^b	10.28 ± 3.18^b	5.90 ± 2.05^a	5.83 ± 2.02^a
BOD5 (mg/L)	7.49 ± 2.26^c	4.26 ± 0.81^b	4.21 ± 1.44^b	3.13 ± 1.15$^{a\,b}$	2.91 ± 0.95^a

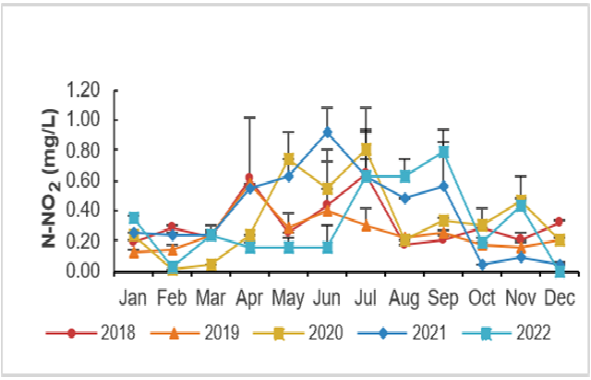
TSS (mg/L)	16.21 ± 3.5^{a}	22.15 ± 4.93^{a}	20.80 ± 9.38^{a}	23.60 ± 10.95^{a}	23.60 ± 9.57^{a}
Phytoplankton (cell/L)	$4.241.148 \pm 2.518.923^{b}$	436.024 ± 164.981^{a}	454.569 ± 287.170^{a}	141.066 ± 54.520^{a}	158.744 ± 40.586^{a}
Zooplankton (individual/m^3)	199.488 ± 99.758^{a}	154.785 ± 100.041^{a}	263.437 ± 316.556^{a}	190.401 ± 172.526^{a}	$1.736.750 \pm 3.423.897^{b}$
Aeromonas spp. counts (CFU/mL)	$5.38 \pm 4.39x10^{2a}$	$4.74 \pm 2.75x10^{2a}$	$5.51 \pm 3.45x10^{2a}$	$3.27 \pm 8.20x10^{2a}$	$1.262 \pm 0.989x10^{3a}$
Streptococcus spp. counts (CFU/mL)	$1.66 \pm 2.28x10^{2a}$	$4.6 \pm 8.1x10^{1a}$	$3.5 \pm 5.2x10^{1a}$	$2.353 \pm 4.893x10^{3a}$	$2.066 \pm 5.226x10^{3a}$

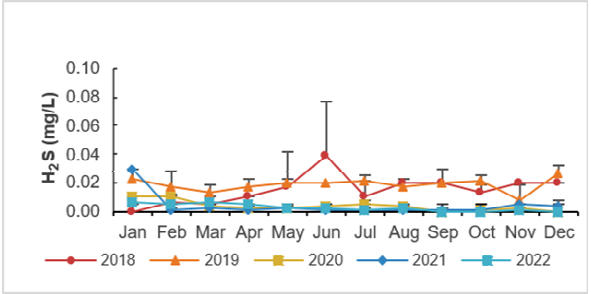
Note: Results are presented as mean ± SD. Means marked with different letters (a, b, and c) indicate significant differences in a parameter across years. Statistical significance is defined as $P < 0.05$.

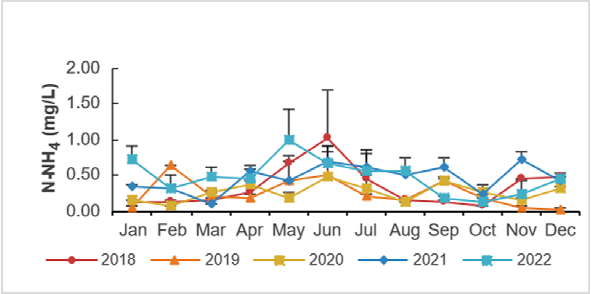


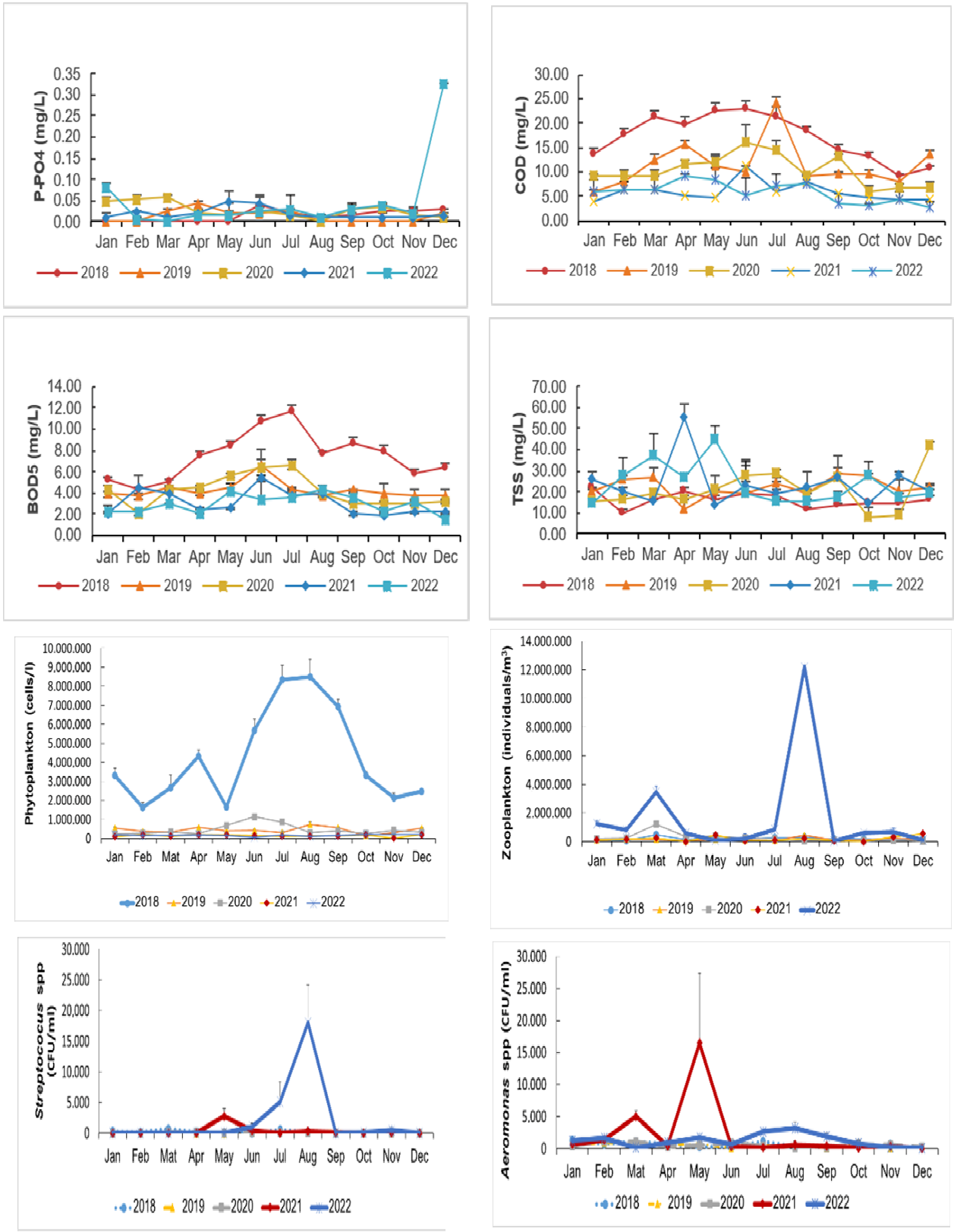












Note: Results are presented as mean ± SD.

Figure 1. Monthly variation of water parameters in Ho Chi Minh Fish Pond from 2018-2022

Water temperature and pH

The annual average water temperatures from 2018 to 2022 were 26.58 ± 3.27°C, 26.06 ± 3.11°C, 26.23 ± 3.67°C, 26.71 ± 3.72 °C, and 25.79 ± 4.55°C, respectively, which were not significantly different ($p>0.05$). The water temperature was seasonally fluctuating. The water temperature was lowest in January (21.42 ± 2.49°C) and highest in June (30.85 ± 0.45°C) (Figure 1). The great depth of the water column (2.36 m) minimized the sudden change in water temperature.

The annual average pH values from 2018-2022 were 7.72 ± 0.18, 7.65 ± 0.21, 7.64 ± 0.21, 7.64 ± 0.14, and 7.84 ± 0.37, respectively (Table 1). The pH value in 2022 was significantly higher than that in 2019, 2020 and 2021 ($p<0.05$). pH was lowest in January (7.60 ± 0.16) and highest in September (7.94 ± 0.37). pH range of 7.0-8.0 is ideal for tilapia and common carp [27,28]. The stable pH levels in the Ho Chi Minh Fish Pond created favorable conditions for the fish and water environment [29].

Dissolved Oxygen (DO)

The average DO levels increased over the years, with average values being maintained above 3 mg/L. The average DO concentration in 2022 was 5.33 ± 1.33 mg/L, which was higher than in 2018, 2019, 2020, and 2021 ($P<0.05$). AWMS recorded several instances of DO levels falling below 3 mg/L. The percentages of DO levels below 3 mg/L in 2018, 2019, 2020, and 2021 were 45.1%, 23.3%, 21.0%, and 31.4%, respectively. In 2022, the percentage of DO levels below 3 mg/L was 16.8%, significantly decreased compared to 2021. The major renovation of the pond in October 2021, with the removal of sediment, increased the DO levels. It is known that the sediment layer regulates the productivity of the water layer and oxygen consumption [30]. Clearing sediment from the pond eliminated organic matter, a crucial oxygen consumer. DO levels did not exhibit significant fluctuations across months, ranging from 3.36 ± 1.16 mg/L in April to 4.69 ± 2.49 mg/L in September. The intensive aeration schedule and low phytoplankton density were the factors that mitigated the seasonal impact on DO levels. In this study, the DO level was correlated to COD (R=-0.45), BOD5 (R=-0.41), H_2S (R=-0.35), and phytoplankton (R=-0.31). Wang *et al*. (2013) [31] also reported the negative correlation between DO and COD, BOD5, and H_2S. Phytoplankton consumes oxygen during the night, leading to a significant decrease in DO in the morning. In this study, phytoplankton was sampled at 7:00 a.m with low a DO level, explaining the observed negative correlation.

Ammonium nitrogen (N-NH_4) and Nitrite nitrogen (N-NO_2)

The annual average N-NH_4 concentrations from 2018-2022 were 0.34 ± 0.29 mg/L, 0.25 ± 0.20 mg/L, 0.25 ± 0.13 mg/L, 0.46 ± 0.20 mg/L, and 0.47 ± 0.26 mg/L, respectively. The N-NH_4 levels showed a decreasing trend from 2018 to 2020 and then increased in 2021 and 2022. N-NH_4 concentrations were consistently below the allowable limit value according to the Vietnam regulation for water quality in tilapia pond culture (1 mg/L). The concentrations of N-NH_4 were 0.54 ± 0.31 mg/L in May and 0.67 ± 0.22 mg/L in June, respectively, significantly higher than those observed in the remaining months ($p<0.05$).

The annual average concentrations of $N\text{-}NO_2$ were 0.32 ± 0.17 mg/L, 0.26 ± 0.13 mg/L, 0.35 ± 0.26 mg/L, 0.39 ± 0.28 mg/L, and 0.35 ± 0.24 mg/L, respectively. Average $N\text{-}NO_2$ concentrations showed an increasing trend from February (0.14 ± 0.12 mg/L) to July (0.60 ± 0.28 mg/L), then decreased in the following months. The increases of $N\text{-}NH_4$ and $N\text{-}NO_2$ were attributed to the accumulation of fish excrete and feed residue combined with elevated decomposition processes during high-temperature periods. Despite regular oxygen supply from the aeration system and the use of probiotic products to facilitate the conversion of $N\text{-}NO_2$ to $N\text{-}NO_3$, the $N\text{-}NO_2$ levels in the pond have remained consistently high over the years and exhibit similar fluctuations from year to year. The presence of $N\text{-}NO_2$ in the pond depends on several factors, such as temperature, pH, DO, and the content of organic carbon [32]. Elevation of temperature speeds up organic compound decomposition, leading to increased $N\text{-}NO_2$ levels in April, May, June, and July. Additionally, runoff from the nearby garden during heavy rain events introduced nitrogen sources to the pond, evident in the high levels of these compounds during these rainy months. Although high $N\text{-}NO_2$ levels were detected, we did not observe any adverse effects on fish. Previous studies reported that the LC50 for $N\text{-}NO_2$ in fish can go up to 120.84 mg/L [32]. According to Somerville *et al*. (2014) [33], the allowable $N\text{-}NO_2$ for warm-water fish is lower than 1 mg/L, which is higher than our result.

Hydrogen sulfide (H_2S)

H_2S is commonly present in aquatic environments under anaerobic conditions and is highly toxic to aquatic organisms. As temperature and pH increase, the proportion of H_2S in the total sulfide content decreases, and vice versa [34]. The annual average H_2S levels from 2018 to 2022 were 0.015 ± 0.01 mg/L, 0.019 ± 0.01 mg/L, 0.004 ± 0.003 mg/L, 0.004 ± 0.01 mg/L, and 0.003 ± 0.003 mg/L, respectively. H_2S levels in 2020, 2021, and 2022 were significantly lower than those in 2018 and 2019 ($P<0.05$). There was no significant difference between months ($P>0.05$). H_2S levels remained low and had no adverse effects on fish health.

Chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5)

From 2018 to 2022, COD values showed a decreasing trend. The COD values in 2021 (5.90 ± 2.05 mg/L) and 2022 (5.83 ± 2.02 mg/L) were significantly lower than those in 2018 (17.11 ± 4.69 mg/L), 2019 (11.39 ± 4.86 mg/L), and 2020 (10.28 ± 3.18 mg/L) ($P<0.05$). COD values tended to increase from January (7.76 ± 3.86 mg/L) to July (14.67 ± 8.22 mg/L) and then showed a decreasing trend toward December (7.63 ± 4.57). The annual average BOD_5 values from 2018 to 2022 were 7.49 ± 2.26 mg/L, 4.26 ± 0.81 mg/L, 4.21 ± 1.44 mg/L, 3.13 ± 1.15 mg/L, and 2.91 ± 0.95 mg/L, respectively. BOD_5 values in 2021 and 2022 were lower than those from 2018 to 2020 ($P<0.05$) and remained below 4 mg/L. BOD_5 increased from February (3.40 ± 1.15 mg/L) to July (6.57 ± 3.39 mg/L) and then showed a decreasing trend.

COD and BOD_5 fluctuations followed seasonal and temperature patterns. Higher temperatures promote the decomposition of organic matter and increase oxygen consumption in the pond. Previous research indicated that only about 25% of

dry feed is converted into fish meat, with the remaining 75% released into the environment as excess feed and fish waste [35]. Under optimal oxygen conditions, the breakdown of organic matter accelerates with the elevation of temperature, leading to a decrease in waterborne organic content. However, in aquaculture ponds, part of oxygen is utilized by the fish, leaving the rest for the decomposition of organic matter. The nutrients released during organic matter breakdown can stimulate algae growth. Correlation analysis revealed positive correlations between COD and algae density ($R= 0.63$, $P<0.05$), H_2S ($R=0.44$, $P<0.05$), and BOD_5 ($R=0.63$, $P<0.05$) (SM 2). This finding aligns with Lau *et al*. (1995) [36], suggesting that the interaction between algal and bacterial cells could enhance the simultaneous removal of nitrogen, phosphorus, and organic matter. COD fluctuations can alter the composition and increase the development of bacteria in the water, as well as the formation of toxic gases in the pond. Therefore, effective COD control, especially during the summer months, is important for maintaining the water quality. Barbooti (2017) [37] noted that within the temperature range of 22-32.5°C, higher temperatures lead to an increased activity of bacteria breaking down organic matter. Nitrogen and phosphorus are factors directly influencing the increase in BOD_5. Conversely, the abundance of nutrients and organic matter creates suitable conditions for microbial growth in the pond.

Total suspended solids (TSS) and phosphorus ($P-PO_4$)

From 2018 to 2022, the average TSS levels were 16.21 ± 3.50 mg/L, 22.215 ± 4.93 mg/L, 20.80 ± 9.38 mg/L, 23.60 ± 10.95 mg/L, and 23.6 ± 9.57 mg/L, respectively. High TSS levels were attributed to the presence of large carp in the pond [38,39], affecting the aesthetics and visibility of the pond. Previous studies reported that cyprinid fish (carp, barbell, tench) are more tolerant to higher levels of TSS than salmonid fish [40]. In this study, TSS levels did not adversely affect the health of fish.

The annual average $P-PO_4$ concentrations were 0.015 ± 0.01 mg/L, 0.013 ± 0.02 mg/L, 0.028 ± 0.02 mg/L, 0.019 ± 0.01 mg/L, and 0.022 ± 0.02 mg/L, respectively. $P-PO_4$ in aquaculture environments tends to precipitate due to suspended particles and settle at the bottom of the pond [41]. This explains the low $P-PO_4$ concentration in water.

Phytoplankton and zooplankton

From 2018 to 2022, six phyla of algae were detected, including Bacillariophyta, Chlorophyta, Euglenophyta, Bacillariophyta, Ochrophyta, and Cyanobacteriophyta. Among these, Chlorophyta was dominant in both composition and density. Our unpublished data reported 49 species of chlorophyte compared to Bacillariophyta (8 species), Euglenophyta (18 species), Bacillariophyta (8 species), Ochrophyta (1 species), and Cyanobacteriophyta (16 species). This finding is consistent with previous studies, that Chlorophyta is one of the most species-rich of the algal groups, with a total of 500 genera and an estimated 14,000 green algae species [42]. The annual average phytoplankton density were 4,241,148 ± 2,518,923 cells/l, 436,024 ± 164,981 cells/l, 454,569 ± 287,170 cells/l, 141,066 ± 54,520 cells/l, and 158,744 ± 40,586 cells/l, respectively. The algae density from 2019 to 2022 significantly decreased compared to 2018 ($p<0.05$). Zahangir *et al*. (2021) [29]

suggest that in intensive ponds, top-down effect is an important strength in phytoplankton communities. A low density of phytoplankton is important for maintaining high phytoplankton diversity and water stabilization in aquaculture. Moreover, low algae density contributes to the visual attractiveness of the fishpond. Phytoplankton densities were typically higher in June, July, August, and September. High temperatures speed up the mineralization rates of the organic matter that provides nutrients for algae growth [43].

There were four groups of zooplankton in the pond, including Nauplius, Copepoda, Rotatoria, and Cladocera. Among these, Rotatoria group was predominant. The annual average densities of zooplankton from 2018-2022 were 199,488 ± 99,758 individuals/m³, 154,785 ± 100,041 individuals/m³, 263,437 ± 316,556 individuals/m³, 190,401 ± 172,526 individuals/m³, and 1,736,750 ± 3,432,897 individuals/m³, respectively. The zooplankton density showed an increasing trend from 2019 to 2022 and reached a peak in 2022 ($P<0.05$). The density of zooplankton significantly increased in August (2,642,800 ± 5,353,717 individuals/m³) ($P<0.05$). This finding indicates the seasonal pattern in zooplankton development. The shifts observed in August align with the stages of zooplankton development, coinciding with the rapid growth of phytoplankton during this period. This surge in phytoplankton serves as a substantial food source for the flourishing zooplankton population during these months.

***Aeromonas* spp. and *Streptococcus* spp. counts**

The annual average densities of *Aeromonas* spp. count from 2018-2022 were 5.38 ± 4.39 $x10^2$ CFU/mL, 4.74 ± 2.75$x10^2$ CFU/mL, 5.51 ± 3.45 $x10^2$ CFU/mL, 3.27 ± 8.20 $x10^2$ CFU/mL, and 1.26 ± 0.99$x10^3$ CFU/mL, respectively. This result magnification (10^1-10^3 CFU/mL) was similar to the *Aeromonas* spp. count of a previous study that reared tilapia for 120 days in [44]. The *Aeromonas* spp. count showed a similar pattern between 2018, 2019, and 2020, and a significant increase in 2021 and 2022 ($P<0.05$). The *Aeromonas* spp. count was typically high from May to August. Previous study reported that the density of *Aeromonas hydrophila* positively correlated with temperatures, with optimal growth occurring at 30°C [45]. In our study, *Aeromonas* spp. positively correlated to phytoplankton and zooplankton. This result agrees with previous studies that reported zooplankton carried *Aeromonas* spp. much more than other materials such as carcasses and fecal pellets harbored [46, 47].

Streptococcus spp., including *Streptococcus agalactiae* and *Streptococcus iniae*, have recently resulted in significant economic losses in tilapia, eels, mullet, bass, and catfish [48-50]. A strong correlation between the presence of *S. agalactiae* and fish mortalities was reported [51]. In this study, the annual average of *Streptococcus* spp. count ranged from 3.5 ± 5.2$x10^1$ to 2.35 ± 4.89$x10^3$ CFU/mL over the five-year period. From 2018 to 2020, the *Streptococcus* spp. counts narrowly fluctuated. In 2021 and 2022, there were increases in the *Streptococcus* spp. count; however, no statistically significant difference was observed ($P>0.05$). The abrupt spikes in March, May, and August

in both 2021 and 2022 resulted in an overall increase in the total counts of *Streptococcus* spp. count during these two years and specifically in these three months over the five-year period. The 5-year average *Streptococcus* bacteria in March, May, and August were 1.21 ± 2.16 x10^3, 3.30 ± 7.37 x10^3, and 3.77 ± 7.96 x10^3 CFU/mL, respectively. In this study, *Streptococcus* spp. were positively correlated to nitrite (R=0.28, P<0.05), *Aeromonas* spp. (R=0.66, p<0.05), and zooplankton (R=0.68, p<0.05). *S. agalactiae* experienced a significant increase in population when the temperature ranged between 25 to 30°C [51, 52]. This temperature range was observed in the months of March, May, and August. The concurrent presence of favorable conditions, such as the availability of zooplankton and specific physico-hydrochemical factors, further contributed to the heightened *Streptococcus* spp. counts during these months.

Seasonal water environment changes

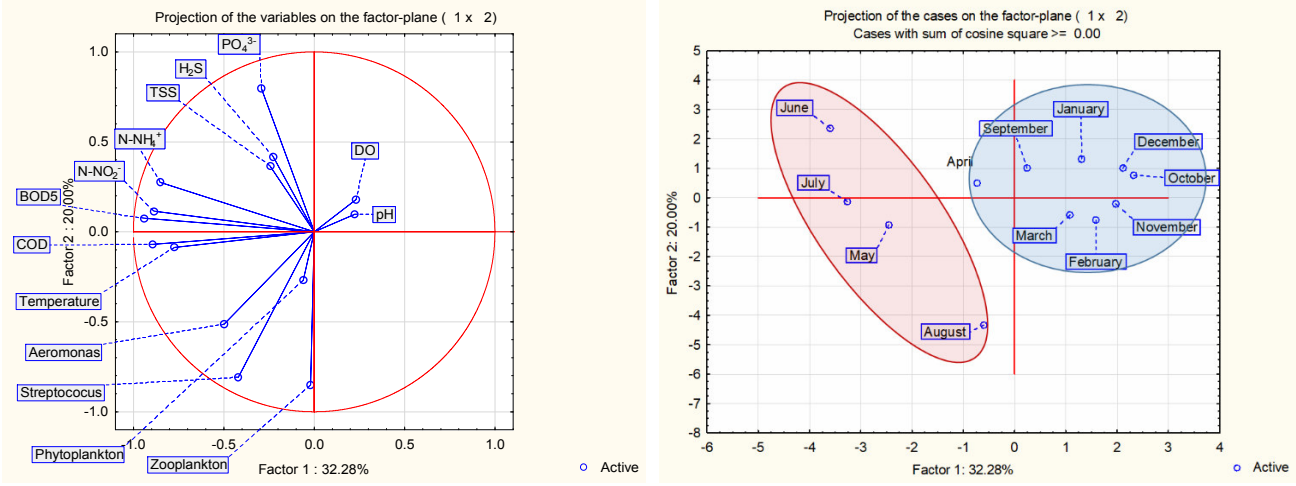


Figure 2. Principal component analysis of all environmental parameters over five years. Note: Principal component analysis (PCA) projection of cases (A) and variables (B) on factor 1 vs. 2. Variables are average values of parameters according to months in five years

The Principal Component Analysis (PCA) result shows that the water environment of Ho Chi Minh Fish Pond had significant seasonal variations (Figure 2A). These are apparently separated in the projection of factor plane 1 vs. 2, reflecting the seasonal effect (Figure 2B). These two PCs explain 52.28% of the total variance. Factor one was positively correlated to temperature (0.77), COD (0.89), N-NO_2^- (0.89), BOD_5 (0.94), *Aeromonas* spp. (0.59), *Streptococcus* spp. (0.42), reflecting the condition in May, June, July, and August (Figure 2A). A previous study also reported seasonal changes with the decrease of DO and elevation of COD and BOD in summer [53]. Li *et al*. (2013) [54] indicated that rising temperatures lead to an increase in ammonia, nitrate, total nitrogen, COD, and phosphate, and a decrease in DO. It has been reported that an increase in temperature leads to higher oxygen consumption in fish [55]. Additionally, an increase in temperature can accelerate the decomposition of organic matter,

potentially leading to the release of inorganic nutrients and toxic gases. Factor two reflects the condition of Winter and Autumn with better oxygen conditions. This result is consistent with previous studies, which suggest that oxygen levels are highest during winter [56] Our results underscore the importance of paying special attention to water quality management during the summer season. Controlling feeding to minimize uneaten feed may help reduce organic pollution. Additionally, adding microbial water treatment products when necessary can help improve water quality.

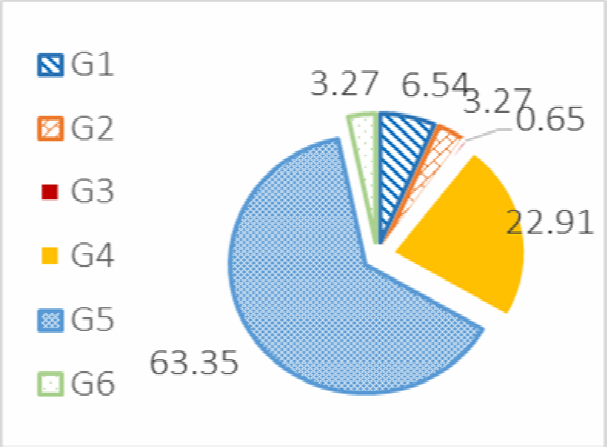
3.2. Fish population structure and co-occurrence network

The fish population structure in the pond was categorized into six groups, representing a dynamic ecosystem with diverse feeding behaviors and habitat layers (Figure 2). These groups exert both direct and indirect effects on key ecosystem parameters, including plankton density, organic detritus turnover, plankton regulation, and predator-prey interactions. The structural model of fish population includes:

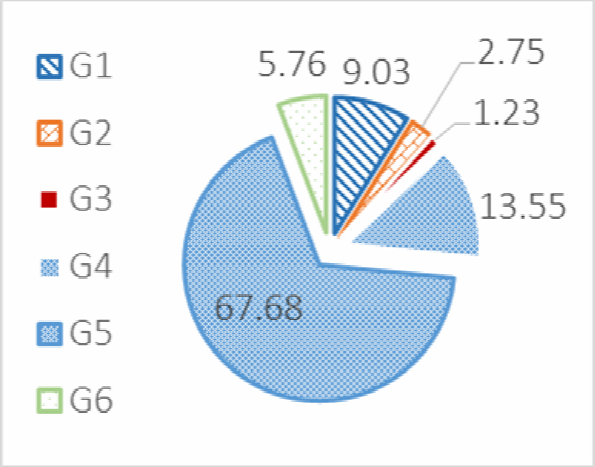
- **Group 1 (G1):** Consisting of silver carp (*Hypophthalmicthys molitrix*) and bighead carp (*Aristichthys nobilis*), which live in the surface water layer and influence plankton density and water clarity [57, 58].
- **Group 2 (G2):** Consisting of mud carp (*Cirrhinus molitorella*), rohu (*Labeo rohita*), and mrigal carp (*Cirrhinus mrigala*), which contribute to detritus recycling in the middle and bottom layers.
- **Group 3 (G3):** Consisting of grass carp (*Ctenopharyngodon idellus*) impacting aquatic plant biomass.
- **Group 4 (G4):** Consisting of climbing perch (*Anabas testudineus*), tilapia (*Oreochromis niloticus*), and barbel chub (*Squaliobarbus curriculus*),
- **Group 5 (G5):** Consisting of common carp (*Cyprinus carpio*), crucian carp (*Carassius auratus*), and black carp (*Mylopharyngodon piceus*), which inhabit both the bottom and middle water layers. G4 and G5 groups function as omnivores, playing a vital role in organic material cycling and nutrient redistribution [59-62].
- **Group 6 (G6):** Consisting of Hong Kong catfish (*Clarias fuscus*), amur catfish (*Silurus asotus*), blotched snakehead (*Channa maculate*), North African catfish (*Clarias garieprinus*), and turtles, which inhabit the bottom water layer and play a critical role in controlling the population of small fish, particularly self-reproducing tilapia [63].

The population structure of the fish was distributed among groups G1, G2, G3, G4, G5, and G6 with the respective proportions of 6.54%, 3.27%, 0.65%, 22.91%, 63.35%, and 3.27% in terms of fish numbers (Figure 3A) and 9.03%, 2.75%, 1.23%, 13.55%, 67.68%, and 5.76% in terms of biomass (Figure 3B). To our knowledge, this is one of the few studies experimenting with the composition of polyculture fish populations involving a large number of species (16 species). In our study, due to the historical and aesthetic characteristics of the pond, common carp accounted for the largest proportion. We adjusted the quantities of other species to maintain nutritional balance and optimize feed utilization within the pond. The G4 group ranked second in proportion and played a key role in processing waste and leftover

feed from G5 species. Although G6 and G2 species were less abundant, they were crucial for controlling tilapia reproduction and decomposing organic residues at the pond bottom. The G1 group was essential in regulating algae density within the pond. According to annual records, 2-5 individual fish, mainly female common carp, died during the spawning season with no apparent signs of disease. The dead individuals and sampled disease-tested fish were replaced by new individuals (free of KHV and SVC) in the same quantity each year.



A, Percentage of groups by number of fish (%)



B, Percentage of groups by weight (%)

Code	Groups
G1	Group of fish that eat plankton and inhabit the upper water layer (silver carp, bighead carp)
G2	Group of fish that eat organic detritus and inhabit the middle and bottom water layers (mud carp, rohu, mrigal)
G3	Group of fish that eat plants and inhabit the middle and upper water layers (grass carp)
G4	Group of fish that are omnivorous, favor plants, and inhabit the bottom and middle water layers (climbing perch, tilapia, Barbel chub)
G5	Group of fish that are omnivorous, favor animals and inhabit the bottom and middle water layers (common carp, crucian carp, black carp)
G6	Group of fish that eat small fish and other animals and inhabit the bottom water layer (Hong Kong catfish, amur catfish, blotched snakehead fish, North African catfish, turtle)

Figure 3: Fish percentage by number and weight in Ho Chi Minh Fish Pond from 2018 to 2022

The co-occurrence network identified G5 as a keystone group, bridging multiple trophic levels and significantly contributing to ecosystem stability. Positive associations between G5 and G4, as well as between G1 and G2, highlight their complementary roles in nutrient cycling and plankton regulation. Common carp and silver carp exhibit a complementary nutrient cycling relationship within the ecosystem. Common carp stir up sediment, recycling nutrients into the water column, which stimulates phytoplankton production. These phytoplankton serve as a food source for silver carp. Conversely, the fecal pellets produced by silver carp, enriched with partially digested phytoplankton, are consumed by common carp, allowing them to utilize algae they would otherwise be unable to digest [64]. Bui *et al*. (2023) [65] reported that carp species with different feeding habits help manage microalgal size and composition. Negative associations between G6 and the smaller omnivorous groups (G4 and G5) reflect predator-prey dynamics. In contrast, G3 showed weak network interactions due to its scarce presence, linked to limited vegetation in the pond. Previous research has reported that the proportions of species in polyculture systems can influence the yield of each species. For instance, when Nile tilapia, common carp, and silver carp are cultured together, the yield of common carp and silver carp tends to decrease as the proportion of Nile tilapia increases [66]. Therefore, in our study, biological control of the tilapia population received significant attention and achieved remarkable effectiveness. Another study found that a composition of 15% common carp, 30% grass carp, 5% silver carp, 10% bighead carp, 20% jundia, and 20% Nile tilapia produced the best farming results [67]. Nile tilapia grew better in the absence of common carp, whereas common carp performed better when Nile tilapia were present.

3.3. Pathogens and Fish Health Status

3.3.1. Physiological state of fish

The data on the number of fish species, average weight, and condition factor in 2021 are presented in table 2. The primary objective is to maintain optimal fish health rather than maximizing production, leading to the omission of fish weight gain considerations. Fish health was evaluated based on their appearance and the condition factor index (CF), which reflects the physiological state of fish in relation to their well-being [68]. The CFs of 16 species exceeded 1, except for amour catfish (0.97), blotched snakehead (0.95), and black carp (0.92). Previous studies have reported cases of CF lower than 1 for these species [69, 70] (Table 2). In this study, the CFs for these three species were approximately 1, indicating a satisfactory condition. The low CF of these species may be linked to the low abundance of natural food, such as snails and small fish. The condition factor of common carp ranged from 1.48 to 2.29. According to Tessema *et al*. (2020) [71], CF > 1 signifies the good condition of common carp. This result is consistent with a prior study reporting common carp CF ranging from 1.31 to 2.25 [72]. The CF for rohu, mrigal, and mud carp was 1.91, 1.76, and 1.12, respectively, reflecting a good condition, as documented by previous

studies [73, 74]. Grass carp's CF was 1.09, which was lower compared to the findings of Chitrakar and Parajuli (2017) [75], with CF ranging from 0.90-3.13. The litter plant feed in the pond may explain this discrepancy. The CF of tilapia was 2.05, aligning with the range reported in previous studies [76]. These results illustrate that the population structure in Ho Chi Minh Fish Pond was appropriate.

Table 2. Average weight and number of fish in Ho Chi Minh Fish Pond in 2021

	English name	Latin name	Number of fish (individual)	Average weight (kg)	Total weight (kg)	% by number of fish	% by total fish weight	CF
1	Silver carp	*Hypophthalmic hthys molitrix*	50	2.88 ± 0.38	144	3.27	2.82	1.88 ± 0.16
2	Bighead carp	*Aristichthys nobilis*	50	6.30 ± 1.05	315	3.27	6.17	1.34 ± 0.04
3	Mud carp	*Cirrhinus molitorella*	10	2.79 ± 0.68	27.9	0.65	0.55	1.12 ± 0.06
4	Mrigal	*Cirrhinus mrigala*	20	2.81 ± 0.67	56.2	1.31	1.10	1.76 ± 0.09
5	Rohu	*Labeo rohita*	20	2.78 ± 0.69	55.6	1.31	1.09	1.91 ± 0.08
6	Grass carp	*Ctenopharyngo don idellus*	10	6.23 ± 1.79	62.3	0.65	1.22	1.14 ± 0.15
7	Climbing perch	*Anabas testudineus*	20	0.022 ± 0.004	0.44	1.31	0.01	2.07 ± 0.09
8	Nile Tilapia	*Oreochromis niloticus*	300	2.04 ± 0.49	612	19.63	11.98	2.05 ± 0.13
9	Barbel Chub	*Squaliobarbus curriculus*	30	2.35 ± 0.45	70.5	1.96	1.38	1.54 ± 0.14
10	Vietnam Common Carp	*Cyprinus carpio*	20	3.59 ± 0.72	71.8	1.31	1.41	1.45 ± 0.19
	Hungarian Hybrid Selective Common carp		200	3.68 ± 0.63	736	13.09	14.41	2.29 ± 0.31
	Hungarian Scale Common carp		60	3.57 ± 0.58	214.2	3.93	4.19	1.95 ± 0.24
	Hungarian Mirror Common carp		5	3.61 ± 0.63	18.05	0.33	0.35	1.98 ± 0.25

	Indonesian Yellow Common carp		650	3.63 ± 0.60	2.359	42.54	46.17	1.48 ± 0.22
11	Crucian carp	*Carassius auratus*	30	0.32 ± 0.07	9.7	1.96	0.19	1.54 ± 0.20
12	Black carp	*Mylopharyngod on piceus*	3	9.6 ± 1.2	28.7	0.2	0.56	0.92 ± 0.08
13	Hong Kong Catfish	*Clarias fuscus*	10	2.29 ± 0.41	22.9	0.65	0.45	2.41 ± 0.21
14	Amur Catfish	*Silurus asotus*	10	9.90 ± 3.84	99	0.65	1.94	0.97 ± 0.11
15	Blotched snakehead	*Channa maculata*	20	3.61 ± 0.58	72.2	1.31	1.41	0.95 ± 0.16
16	North African catfish	*Clarias gariepinus*	10	9.86 ± 1.57	98.6	0.65	1.93	1.45 ± 0.09
17	Turtle	Trionychidae	1	35	35	0.07	0.69	
Total					5.109.1	100	100	

3.3.2. Occurrence of fish pathogens

In this study, fish were not sampled monthly. Samples were taken only when fish exhibited signs of disease/abnormal health. The pathogen infection rates are presented annually (Figure 4).

Bacterial pathogens

Aeromonas hydrophila, *Plesiomonas shigelloides*, and *Pseudomonas fluorescens* are commonly found in freshwater and gut flora of fish [77]. These agents pose potential threats to fish in aquaculture. The risk of outbreaks rises with water pollution, and fish stress increases [78, 79]. From 2018 to 2022, seven bacterial species were identified in liver and kidney of fish, including *A. hydrophila*, *Aeromonas trota*, *Aeromonas popoffii*, *Aeromonas allosaccharophila*, *Aeromonas sobria*, *P. shigelloides*, and *P. fluorescens* (Figure 4A). Among them, *A. hydrophila* was the most frequently detected bacterium, with a 5-year average infection rate of 19.22%. The next most frequently detected bacteria were *P. shigelloides* (10.48%), *A. trota* (4.00%), *P. fluorescens (2.54%)*, *A. allosaccharophila* (2.4%), *A. popoffii* (0.8%), and *A. sobria* (0.8%).

A. hydrophila was detected with the annual average infection rate ranging from 0-66.7% of tested fish. *A. hydrophila* is a freshwater, facultative anaerobic, chemo-organoheterotrophic bacterium that distresses fish with gastroenteritis, septicemia, and causes a disease known as motile aeromonas septicemia [80-82]. Other *Aeromonas* species, including *A. allosaccharophila* (12.0% infected in 2020), *A. trota* (20.0% infected in 2021), *A. popoffii* (4.0% infected in 2022), and *A. sobria* (4.0% infected in 2022), were detected with a low frequency. *A. allosaccharophila* is a gram-negative, rod-shaped, non-spore-forming bacterium that causes disease and death in aquaculture

species. Shao *et al*. (2023) [83] reported the LD50 of *A. allosaccharophila* was 3.65 $\times 10^5$ CFU/mL on leech. However, little information about the effect of this species on fish was reported. *A. popoffii* has most of characteristic properties known in *Aeromonas* spp. [84]. The occurrence of *A. popoffii* was previously reported in sediment in tilapia cultured pond [85]; however, no serious disease outbreak was reported. *A. sobria* causes disease through its motility [79] and was responsible for significant losses in tilapia production, particularly in the over-wintering stage [86]. *A. trota* was previously detected in tilapia; however, the effect of this bacterium on fish was not reported in detail [87].

P. shigelloides is a motile, facultative anaerobic, gram-negative aquatic bacterium recently recognized as a potential aquatic animal pathogen. *P. shigelloides* shows elastolytic activities, which may be involved in connective tissue degradation. *P. shigelloides* was reported to be related to the mortality of *Ctenopharyngodon idellus* [88] and *Oreochromis nilotica* [89]. *P. fluorescens* was often associated with skin and fin disease in farmed *C. carpio* [90]. In this study, the second most frequently detected bacterium was *P. shigelloides*, suggesting the high risk of this bacterium on fish; however, no apparent damage was observed.

Despite the high density of *Streptococcus* spp. in the water in 2021 and 2022. *Streptococcus* spp. were not detected in the livers and kidneys of the collected fish. Pathogenesis can be influenced by some factors including the virulence of the agent, the environmental stressors, and the susceptibility of the host [91]. In this study, good health conditions and low environmental stress are the key factors explained for the *Streptococcus* spp. result.

Parasitic pathogens

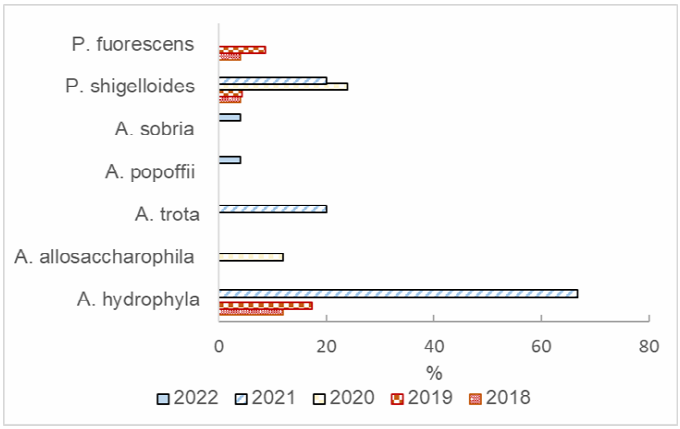
Parasites are commonly found in both natural and farmed aquatic animals [92]. They cause mechanical damage (fusion of gill lamellae, tissue replacement), physiological damage (cell proliferation, immunomodulation, detrimental behavioral responses, altered growth), and reproductive damage [93]. Parasites can cause significant damage to fish, especially at fingerling stages [94].

From 2018 to 2022, four parasites and one parasitic fungus on fish were observed, including *Dactylogyrus* sp., *Trichodina* sp., *Epistylis* sp., *Myxobolus* sp., and *Democystidium* sp. (Figure 4B). *Trichodina* sp. and *Dactylogyrus* sp. were the most frequently detected parasites, with the 5-year average infection rates of 27.80% and 24.56%, respectively. The following parasitic infections were *Dermocystidium* sp. (14.66%), *Epistylis* sp. (11.90%), and *Myxobolus* sp. (0.8%).

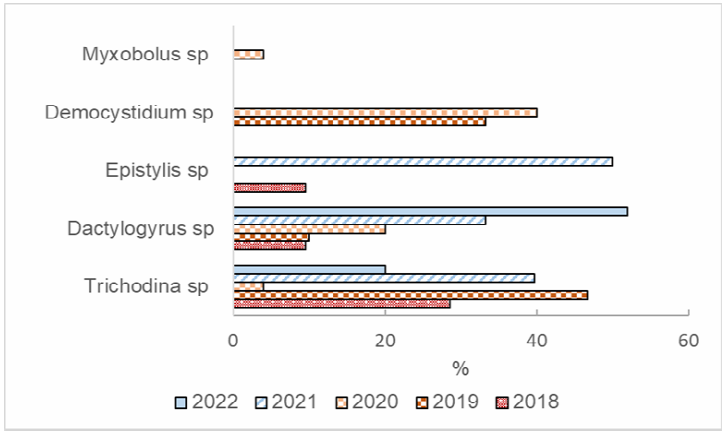
The annual average infection rates of *Dactylogyrus* sp., *Trichodina* sp., and *Dermocystidium* sp. ranged from 9.5-52.0%, 4.0-46.7%, and 0-40.0%, respectively. The average infection rate of *Dactylogyrus* sp. was low compared to the previous study in Macedonia reporting an infection rate of 49.5% from 958 cyprinid specimens [95]. *Dactylogyrus* spp. predominantly affect the gills [96]; however, no serious damage was observed on fish in this study. The infection rate of *Trichodina* sp. was in the same range as previous studies reporting an infection rate

of 22.2% and 49.6% in cultured tilapia and cyprinid ponds [97, 98]. *Dermocystidium* sp. infection rate was high compared to 8.33% in the previous study [99]. *Dermocystidium* spp. are commonly observed on fish, even clinically healthy fish. However, a serious infection can cause damage to gills [100].

Myxobolus parasites are common in the order Cypriniformes. *Myxobolus* spp. cause Myxobolusis disease and lead to a mortality rate of up to 80% [101]. In this study, *Myxobolus* sp. was observed only in 2020, with an infection rate of 4% in common carp. Several species of *Epistylis* were reported to infect the skin, fins, and gills of fish [102]. These parasites cause irritation and inflammation of the epithelium of the host at the point of attachment, which may provide a site for secondary infections of *A. hydrophila* [103]. In this study, we detected *Epistylis* spp. in 2018 and 2021, with the infection rates of 9.5% and 50% in the tested fish, respectively.



A: Bacterial Infections Detected in Fish from Ho Chi Minh Fish Pond from 2018-2020



B: Parasitic Infections Detected in Fish from Ho Chi Minh Fish Pond from 2018-2022

Note: The pathogen infection rates were calculated as the ratio of infected fish to the total number of sampled fish exhibiting disease symptoms within a year.

Figure 4. Pathogen Infection Rates in Fish from Ho Chi Minh Fish Pond from 2018-2022

Viral pathogens

During 5 years, 125 common carp were tested for SVC and KHV. The result shows that all samples were negative for KHV and SVC. An essential factor in safeguarding fish ponds from viruses is the strict control of equipment used in the pond. Additionally, replaced fish released into the pond were thoroughly tested for viruses before introduction. Furthermore, the geographical isolation of the fish pond from other aquatic bodies served as a favorable condition to minimize the risk of virus intrusion.

Relationship of fish pathogens and water environment variables

P. shigelloides was negatively correlated to temperature (R=-0.52, $p<0.05$) and DO (R=-0.55, $p<0.05$) and positively correlated to pH (R=0.57, $P<0.05$). Among the observed bacteria, *P. shigelloides* was the only species that showed correlations with these hydro-physicochemical factors. *A. trota* was positively correlated to *Aeromonas* spp. (R=0.99, $p<0.01$), *Streptococcus* spp. in water (R=1.00, $p<0.01$), and *P. shigelloides* in fish (R=1.00, $p<0.01$). *Epistylis* spp. was positively correlated to *P. fluorescens* (R=1.00, $p<0.01$) and *Dactylogyrus* sp. (R=0.76, $P<0.05$). *Dactylogyrus* sp. was positively correlated to *A. allosaccharophila* (R=1.00, $p<0.01$) and *Epistylis* spp. (R=0.76, $p<0.05$). These results suggest the possibility of pathogen synergistic effect [76]. *A. allosaccharophila* was positively correlated to *Aeromonas* spp. in water (R=0.82, $p<0.05$). *A. sobria* was positively correlated to A. *popoffii* (R=1.00, $p<0.01$). *Myxobolus* sp. was positively correlated to DO (R=0.94, $p<0.05$), which was similarly reported in a previous study [104].

Fish bacteria and parasitic infections typically result from environmental issues, such as subpar water quality, high stocking density, and nutritional deficiencies. Effective water quality management proves more advantageous than administering medication after an infection has already occurred [94]. In our study, upon discovering bacteria and parasites infected fish, measures involving water disinfection and the improvement of fish health through the administration of supplementary vitamins were implemented to reduce the associated risks. The development of disease is a complicated process, dependent not only on bacteria, but also on the immune status of fish, and environmental conditions [79]. During the period from 2018 to 2022, no serious disease outbreaks caused by bacteria were recorded. Previous studies suggested that polyculture with diverse fish species can help limit disease outbreaks. To prevent diseases in carp farming, a notable recommendation is to implement polyculture with fish species that are not susceptible to carp-specific diseases [105]. Our findings reaffirm this argument, as we did not encounter any significant disease issues over five years of practicing polyculture with multiple species.

4. CONCLUSION

A dominant common carp group, combined with 16 other species belonging to plankton, organic detritus, plant consumption, and predator groups, reduces the risk of organic pollution and disease outbreaks.

Greater attention is required during the summer months due to lower water

quality, as indicated by elevated levels of COD, BOD_5, $N\text{-}NH_4$, H_2S, and TSS.

This study highlights the importance of polyculture structure in preserving a historically significant pond. However, management practices and the selection of fish species should take geographical factors into consideration.

In this study, 16 cultured species were selected to reflect and maintain the historical community structure of the study ecosystem. In commercial aquaculture, however, farmers do not necessarily need to culture such a large number of species and may instead focus on a smaller number of high-value species. Nevertheless, the selection of cultured species should represent different trophic levels to promote complementary ecological functions and nutrient recycling within the food web, thereby enhancing resource-use efficiency and improving the overall sustainability of the farming system.

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Data and Code Availability statement:

• [Standardized datatype] data have been deposited at [datatype-specific repository (URL to the repository)] with accession numbers [Database IDs].

• Data included in the article and supplementary materials are referenced in the article.

Declarations

I declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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