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# Effects of thermal stratification and mixing on the vertical distribution of dissolved oxygen in aquaculture ponds

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## **Abstract**

Thermal stratification and mixing in aquaculture ponds can seriously affect the vertical distribution of dissolved oxygen (DO) and result in a lack of DO in ponds. Therefore, revealing the vertical distribution of DO in aquaculture ponds influenced by thermal stratification and mixing can provide a theoretical basis for aquaculture water management. Here, we explore the impacts of thermal stratification and mixing on the vertical distribution of DO in two aquaculture ponds under different weather conditions. The results showed that thermal stratification mainly occurs during the daytime, and mixing occurs at nighttime. Water thermal stratification appears 4 h after sunrise, while mixing occurs 1 h after nightfall. When the Richardson index is less than 0.25, the mixing direction is unstable. In the daytime, the vertical distribution of DO, chlorophyll a (Chl-a), and phytoplankton abundance varied with thermal stratification and mixing. The concentration of DO gradually dropped with increasing water depth during the nighttime. The concentration of DO was lowest in the early morning and peaked in the afternoon. Multiple regression analysis demonstrated that water temperature (WT), Chl-a, and phytoplankton abundance provided the best model for the vertical distribution of DO. Based on our results, DO regulation can provide important insights for aquaculture pond management.

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#### Introduction

Dissolved oxygen (DO) is one of the most important indices of water quality and plays a critical role in maintaining the ecological balance of aquaculture ponds (Nealc et al., 2006; He et al., 2014). The water quality increased with rising DO concentration and deterioration when it was low (Rabalais et al., 2014; Arya and Zhang., 2015), followed by a large number of fish, aquatic plants, and plankton dying, which resulted in economic losses in fishery production and the possibility of a water bloom.

Many environmental factors influence the changes in dissolved oxygen in water (Wang et al., 2012; Liu et al., 2013; Fennel and Testa, 2019), and the most significant factor is mixing. In general, the appearance of a thermogenic layer may reduce the vertical mixing of a water body and obstruct the reaeration process (Du et al., 2018; Wentzky et al., 2019; Jane et al., 2021), which results in a lack of dissolved oxygen in the water body (Breitburg et al., 2018). The vertical distribution of dissolved oxygen shows a sharp decline within 10 m below the surface water (Tian et al., 2022). The mixing regime easily occurred under increased wind speed and rainfall (Okely and Imberger, 2007). It decreases temperature (Sriyasak et al., 2013), which causes the dissolved oxygen to mix and be evenly distributed throughout the water layers (Ni et al., 2016). A previous study showed that the vertical distribution of dissolved oxygen in lakes and reservoirs could become unstable due to seasonal variations in phytoplankton and water temperature (Ouyang et al., 2013; Cavalance et al., 2018). The occurrence of thermal stratification in summer can lead to serious hypoxic conditions in the bottom layer of water, while in winter, the stratification phenomena disappear, followed by a high concentration of dissolved oxygen and a minor differentiation of temperature across the entire water layer (Zhang et al., 2015). Kreling revealed that temperature-induced thermal stratification is the most important factor influencing the vertical distribution of dissolved oxygen (Kreling et al., 2017).

In aquaculture ponds, the vertical distribution of dissolved oxygen shows a similar pattern compared to that in lakes and reservoirs and is closely related to water depth (Jones et al., 2011; Zhang et al., 2014; Oberle et al., 2019). The upper layer usually has high light intensity, phytoplankton photosynthesis, and dissolved oxygen, while the bottom layer showed an opposite trend due to thermal stratification (Moreno et al., 2006). The high temperature may produce a hypoxic environment in aquaculture ponds during summer (Barica et al., 2011). The vertical distribution of dissolved oxygen in water bodies displays evident diurnal variation characteristics. Surface water can absorb solar energy during the daytime and result in stratification. In contrast, at nighttime, the surface water cools down, which promotes the uniform distribution of dissolved oxygen in water bodies (Ryabov et al., 2010). Hypoxia or anaerobic stress that occurs in aquaculture ponds can no longer support the pond's life. The mixing in aquaculture ponds is different from that in lakes and reservoirs due to their lower depth.

Previous work demonstrated that the thermocline might occur every day from May to September and mixed between 20:00 and 22:00 with an average mixing depth of 0.7-1.0 m (Chang et al., 1988). The weak wind speed and decrease in temperature after sunset are the main causes of this phenomenon. Burtle found that the initiation time of mixing in the tropics of low-altitude areas is 22:00-02:00 in winter and 18:00-02:00 in the rainy season, while in high-altitude areas, it is 20:00-22:00 in winter and 14:00-18:00 in the rainy season (Burtle et al., 2014). Different altitudes may influence the mixing time occurring in water bodies. Heavy rainfall after a long time of high temperatures can quickly decrease the surface water temperature of aquaculture ponds, which results in the mixing of water columns (Boontanjai et al., 1989). Hours of sunshine and frontal weather can also lead to mixing (Burtle et al., 2014).

In recent years, many studies have focused on the responses of dissolved oxygen to thermal stratification and mixing in aquaculture ponds (Phan et al., 2008; Sriyasak et al., 2013; Oberle et al., 2019); however, the diurnal vertical distributions of dissolved oxygen in aquaculture ponds are still unclear. Here, we examined the vertical distribution of dissolved oxygen and its relationship with the vertical distribution of environmental variables in aquaculture ponds. Our findings can provide a theoretical foundation for aquaculture water management and protection.

#### **Materials and Methods**

## Study area

The experiments were conducted at two aquaculture ponds: Qixiangju (30°34′88″ N, 112°15′4″ E) and Yaowan (30°26′55″ N, 112°31′57″ E). All of these ponds are located in Jingzhou city, Hubei Province, central China. This region is subject to a north subtropical humid monsoon climate. In summer, the prevailing wind is in the south-east direction. The annual means of historical temperature, total precipitation, sunshine duration, and total radiation are 16.2-16.6 °C, 1100-1300 mm, 1823-1978 h, and 4366.8-4576.2 MJ/m³, respectively. The general characteristics of the study ponds are presented in **Table 1**.

**Table 1** Main characteristics for the two study ponds.

Variables ——	Ponds		
variables	Qixiangju	Yaowan	
Area (m²)	350	200	
Depth (m)	1.2	1.5	
Stocking density (fish/m²)	3	4	
TN (mg/L)	0.64±0.11	0.90±0.08	
TP (mg/L)	0.77±0.01	0.78±0.01	
Retention time (d)	365	365	

## Sampling and sample analysis

Water samples were taken every 2 h from June to July 2021. In each pond, 1 L of water samples was collected every 10 cm for recording biological communities, and 1 L was collected for chemical analyses. Samples were frozen in the dark and delivered to the lab for analysis. Dissolved oxygen (DO) and water temperature (WT) were determined in situ using a multiparameter water quality analyzer. Chl-a were analyzed according to standard methods for water quality monitoring. Data for wind speed (WS) and air pressure (AP) during sampling were provided by the local meteorological bureau (Jingzhou Meteorological Bureau, **Figure 1**).

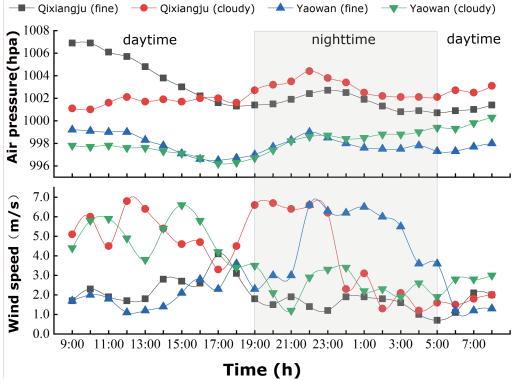


Figure 1 Variations of wind speed and air pressure during sampling.

Phytoplankton samples were fixed with 1.5% acid Lugol's solution and concentrated to 50 ml after sedimentation for 48 h. Samples (0.1 mL) were used to count the phytoplankton species. The counting and calculation were carried out according to Hu and Wei (2006) and Zhang and Huang (1995).

#### Data analysis

We used Excel 2019 to sort the original data. The kriging interpolation method was used to determine the vertical distribution of DO, WT, Chl-a, and phytoplankton abundance. The differences among several environmental variables in aquaculture ponds under different weather conditions were tested using linear regression. The environmental variables were log-transformed to meet the conditions of homogeneity and normality of variance. All statistical analyses were performed using the software packages ArcGIS 10.7, Origin 2018, and SPSS 26.0.

The Richardson index was used to determine the mixing stability index (Chang et al., 1988):

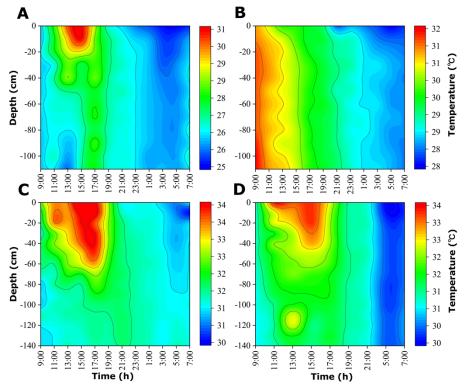
## $Ri=g (dp/dz)/p(du/dz)^2$

where g = gravity acceleration, 980 cm/s; p = water density; z = depth of mixed water layer, cm; u =  $0.48 * w^2$ ; w = wind speed, cm/s, flow rate, cm/s. If Ri > 0.25, the situation is steady. When Ri < 0.25, the water cycle includes vertical and horizontal mixing. The surface water was defined as 0-10 cm below the water surface. The upper-middle water was defined as 0-70 cm below the water surface. The middle and lower water is defined as 70-140 cm below the water surface.

In this study, 'hypoxia' was defined as a DO concentration < 2 mg/L in aquaculture ponds (Burtle et al., 2014).

## Results

The vertical distribution of water temperature in aquaculture ponds
During the sunny days, the stratification of water temperature in the Qixiangju pond occurred
10 h before nightfall and 4 h after sunrise, while mixing occurred 4 h after sunset (**Figure 2A**). Thermal stratification caused a significant difference in temperature between the surface layer and the bottom layer, and the mixing was unstable. During cloudy days, mixing occurred for a long time, even 24 h (**Figure 2B**). Similar to Qixiangju Pond, the time of thermal stratification in the Yaowan pond occurred 11 h before nightfall and 1 h after sunrise, while mixing occurred 3 h after sunset (**Figure 2C and 2D**).



**Figure 2** Vertical distribution of water temperature in Qixiangju Pond during the (A) sunny and (B) cloudy days and Yaowan Pond during the (C) sunny and (D) cloudy days.

The Richardson index in both aquaculture ponds was less than 0.25, indicating that the mixing was unstable during the sunny and cloudy days (**Table 2**). with the main factor being insufficient wind speed, which prevented the mixing from reaching a stable condition.

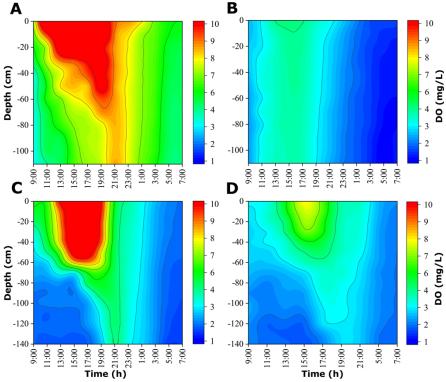
**Table 2** The Richardson stability coefficient in aquaculture ponds under different weather conditions.

Time -	Sunny		Clou	Cloudy	
	Qixiangju	Yaowan	Qixiangju	Yaowan	
9:00			0.088		
11:00			0.054		
13:00			0.219		
15:00			0.059		
17:00			0.015		
19:00			0.248		
21:00	0.002		0.219		
23:00		0.008	0.193	0.005	
1:00	0.002	0.002	0.012		
3:00	0.001	0.001	0.003		
5:00	0.001	0.001	0.001	0.001	
7:00	0.003	0.004	0.001		

The vertical distribution of dissolved oxygen in aquaculture ponds

The vertical distribution of DO in the Qixiangju and Yaowan aquaculture ponds showed a similar pattern (**Figure 3**). During the daytime, the DO concentration shows an apparent vertical distribution feature, with the highest DO concentrated in the middle and upper layers, followed by the middle and lower layers. No stratification of DO occurred in the Yaowan aquaculture pond. During the nighttime, no stratification of DO occurred in either pond due to mixing.

During the sunny days, the average concentration of DO in the upper-middle layers of the Qixiangju and Yaowan ponds ranged from 4.94 to 11.74 mg/L and 2.00 to 11.47 mg/L, respectively. In the middle and lower layers, the average concentration of DO ranged from 3.42 to 6.99 mg/L and 1.70 to 2.82 mg/L, respectively. The concentration of DO showed a significant difference between the surface and bottom layers. During cloudy days, the vertical distribution of DO was similar to that on sunny days. The stratification of DO in ponds was apparent during the daytime. The average concentrations of DO in the upper-middle layers of the Qixiangju and Yaowan ponds ranged from 1.35 to 4.26 mg/L and 2.17 to 5.63 mg/L, respectively. In the middle and lower layers, the average concentration of DO ranged from 1.25 to 4.08 mg/L and 2.04 to 3.10 mg/L, respectively. On sunny days, the concentration of DO was higher than that on cloudy days, and the stratification of DO in ponds was more apparent than that on cloudy days (**Figure 3**).

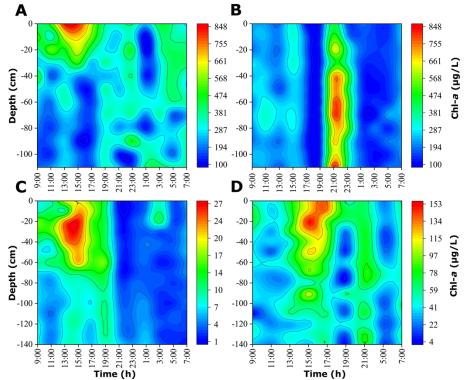


**Figure 3** Vertical distribution of DO in Qixiangju Pond during the (A) sunny and (B) cloudy days and Yaowan Pond during the (C) sunny and (D) cloudy days.

The vertical distribution of Chl-a in aquaculture ponds

The vertical distribution of Chl-a in the Qixiangju and Yaowan aquaculture ponds is shown in **Figure 4**. During the sunny days, the stratification of Chl-a occurred in the two ponds during the daytime. However, on cloudy days, no stratification of Chl-a in the Yaowan pond.

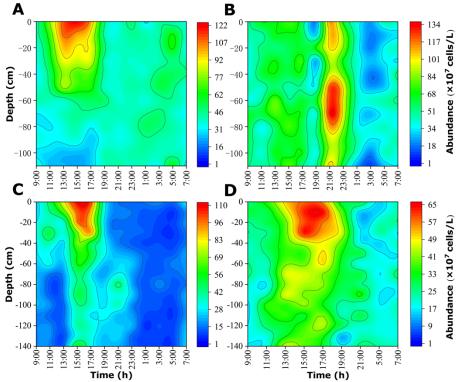
The concentration of Chl-a in the upper-middle layers was relatively high at 15:00 and low in the middle and lower layers. The concentration of Chl-a varied between the surface and bottom layers; with increasing water depth, the concentration of Chl-a rapidly decreased. During the nighttime, no stratification of Chl-a in aquaculture ponds, and the difference in Chl-a concentration between the surface and bottom was small. During the daytime, the concentration of Chl-a was high in the upper-middle layers and decreased with increasing water depth. The difference in Chl-a concentration between the surface and bottom layers was largely due to the thermal stratification in ponds.



**Figure 4** Vertical distribution of Chl-*a* in Qixiangju Pond during the (A) sunny and (B) cloudy days and Yaowan Pond during the (C) sunny and (D) cloudy days.

The vertical distribution of phytoplankton abundance in aquaculture ponds

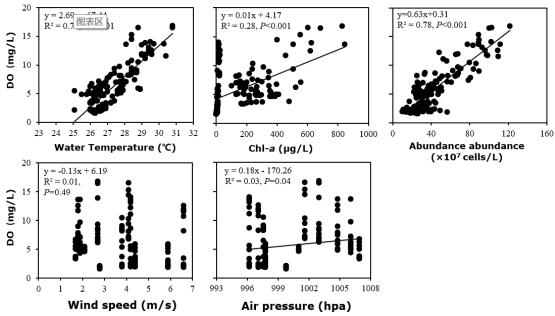
The vertical distribution of phytoplankton abundance was similar to that of Chl-*a* (**Figure 5**). During the daytime, an apparent stratification of phytoplankton abundance occurred in aquaculture ponds. However, no stratification of phytoplankton abundance occurred in aquaculture ponds during the nighttime due to mixing.



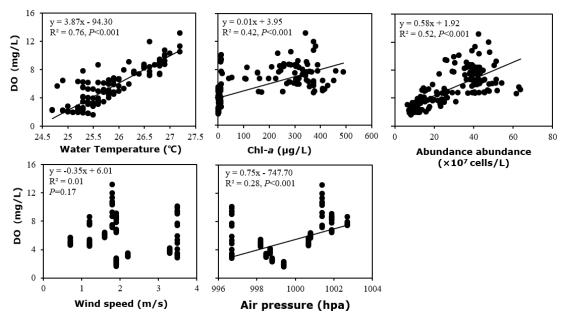
**Figure 5** Vertical distribution of phytoplankton abundance in Qixiangju Pond during the (A) sunny and (B) cloudy days and Yaowan Pond during the (C) sunny and (D) cloudy days.

# Relationships between DO and environmental factors

Linear regression showed that DO increase with WT, Chl-a, phytoplankton abundance, and air pressure during the daytime (**Figure 6**) and nighttime (**Figure 7**) of sunny days. However, no significant relationship was found between DO and wind speed (P<0.05).

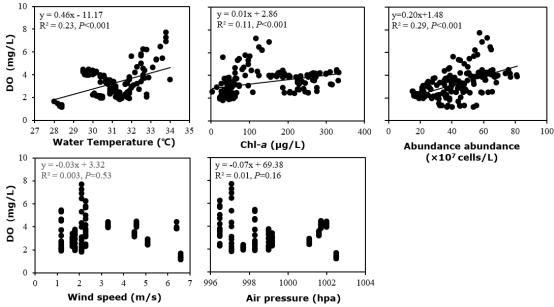


**Figure 6** Correlations between DO and (A) water temperature, (B) Chl-a, (C) phytoplankton abundance, (D) wind speed, and (E) air pressure during the daytime of sunny days (n=162).



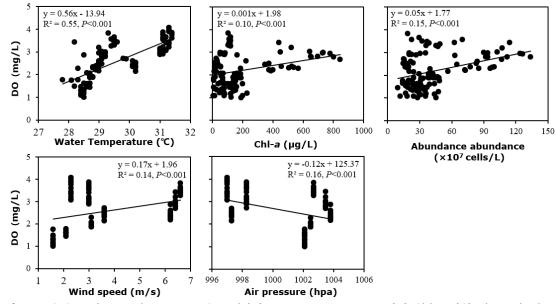
**Figure 7** Correlations between DO and (A) water temperature, (B) Chl-a, (C) phytoplankton abundance, (D) wind speed, and (E) air pressure during the nighttime of sunny days (n=162).

During the daytime on cloudy days, DO showed a significant (P<0.01) positive relationship with WT, Chl-a, and phytoplankton abundance (**Figure8**). However, no significant relationship was found between DO, wind speed, and air temperature (P<0.05).



**Figure 8** Correlations between DO and (A) water temperature, (B) Chl-a, (C) phytoplankton abundance, (D) wind speed, and (E) air pressure during the daytime of cloudy days (n=162).

During the nighttime of cloudy days, DO was significantly (P<0.01) positively correlated with WT, Chl-a, phytoplankton abundance, and wind speed and significantly (P<0.01) negatively correlated with air pressure (**Figure 9**).



**Figure 9** Correlations between DO and (A) water temperature, (B) Chl-a, (C) phytoplankton abundance, (D) wind speed, and (E) air pressure during the night time of cloudy days (n=162).

#### Determinant of DO dynamics

Linear regression models demonstrated that WT, depth, and phytoplankton abundance accounted for most of the variance in the vertical distribution of DO. Interestingly, WT and phytoplankton abundance were significantly (P<0.05) positively correlated with DO, while

water depth exhibited a negative relationship with DO. Furthermore, Chl-a, wind speed, and air pressure also showed significant (P<0.05) positive relationships with DO.

<b>Table 3</b> Linear regression models explaining the dissolved oxygen in aquaculture ponds under					
different weather conditions.					

		Linear model	r²	F
	Daytime	-8.514***+4.897***lgWT-0.064*lgD+0.194***lgP+0.351***lgC	0.87	251.04
Sunny N	Nighttime	-335.907***+5.460***lgWT- 0.125***lgD+0.446***lgP+0.190***lgC+108.337***lgAP+0164*lgWS	0.84	137.23
	Daytime	-9.967*** +4.325***lgWT-0.117***lgD+0.542***lgP	0.65	96.00
Cloudy	Nighttime	-7.324***+4.879***lgWT-0.088***lgD+0.075**lgP+0.263***lgWS	0.78	97.54

Note: \*\*\*P<0.001, \*\*0.001<P<0.01, \*0.01<P<0.05. WT, water temperature; D, depth; C, Chl-a; P, phytoplankton abundance; WS, wind speed; AP, air pressure

#### **Discussion**

Response of dissolved oxygen to environmental factors

In this study, we explored the stratification and mixing of limnological variables over 24 h on sunny and cloudy days. The results showed that thermal stratification mainly occurred during the daytime and mixing mainly occurred during the nighttime, which is consistent with a previous report from shallow aquaculture ponds (Abdelrahman and Boyd, 2016). Thermal stratification usually occurred from 9:00-11:00 A.M. and mixing at 19:00-20:00 P. M, which is earlier than the finding by Chang (1986) in a study on the vertical distribution of dissolved oxygen in aquaculture ponds. This suggests that the rising water temperature may result in an earlier occurrence of thermal stratification (Winder, 2012). According to the Richardson index, mixing could create an unstable state during nighttime, which leads to the exchange of water not only in the upper and lower layers but also in the same layer (Llanillo et al., 2019). Extreme weather (i.e., strong winds) can alter the water temperature in the upper and lower layers and rapidly mix the water column (Cigleneki et al., 2015), followed by long-term and stable mixing.

Our study showed that thermal stratification and mixing could affect the vertical distribution of DO, Chl-a and phytoplankton abundance in aquaculture ponds. This result is consistent with the report by Yu et al., (2017), which supports our findings of the vertical distribution of environmental factors in the water column. The vertical distributions of DO, Chl-a, and phytoplankton abundance in aquaculture ponds showed the same pattern during the daytime and decreased with increased water depth due to thermal stratification. The change in WT in aquaculture ponds resulted in an obvious stratification of DO in the water column (Zhang et al., 2004; Ryabov et al., 2010). During the nighttime, the water exchange between the upper and lower layers becomes more frequent due to the influence of mixing (Wang et al., 2005). In the morning, the DO concentration is low but reaches the highest value at noon and then gradually decreases. The reason for this is that the temperature and light are low in the morning, and phytoplankton consume oxygen, which leads to a low DO environment. Sunshine and high temperature boost the photosynthesis of phytoplankton (Hancke and Glud, 2004; Sekerci and Petrovskii, 2015), followed by a high concentration of DO in the water column.

The vertical distribution of Chl-a and phytoplankton abundance showed a similar pattern during the study periods. The most suitable temperature for phytoplankton growth is 18-25 °C (Hu and Wei, 2006). A high WT may inhibit the growth and reproduction of phytoplankton (Sonk et al., 2013; Zhao et al., 2017) and concentrate at the bottom of the ponds (Lloret et al., 2017), followed by a low Chl-a in the upper layers during the daytime on sunny days.

However, the wind speed was 4.3 cm/s before 19:00 and 6.5 cm/s between 19:00 and 21:00 during the study period. The high wind speed may promote the mixing of the water column (Okely and Imberger 2007; Sriyasak et al., 2013), which drives the phytoplankton in the bottom layer to move to the surface (Siswanto et al., 2005; Hanson et al., 2007). Chl-a is the main pigment for the photosynthesis of phytoplankton and provides DO for aquatic organisms in aquaculture ponds. Nearly 80% of DO comes from phytoplankton (Xu, 2001).

Influence of the thermal stratification and mixing on the vertical distribution of DO Linear regressions showed that WT, Chl-a, phytoplankton abundance, wind speed, and air pressure were significantly (P<0.01) positively correlated with DO, while water depth was significantly (P<0.05) negatively correlated with DO. The water column was classified as the upper, middle, and lower layers due to the vertical change in WT, and thermal stratification would cause an apparent vertical change in DO. However, the stratification of DO may disappear when mixing occurs (Valdespino-Castillo et al., 2014). Warm surface water usually has a high concentration of DO due to sufficient light for photosynthesis, whereas deep ( $\geq$  3 m) cold water shows the opposite trend. A large depth of water may result in a low concentration of DO in the bottom layer, indicating that the variation in WT in the water column may affect the vertical distribution of DO (Zhang et al., 2015).

The concentration of DO varied depending on the weather conditions. During the sunny days, WT, depth, Chl-a, and phytoplankton abundance were the main environmental factors affecting the concentration of DO in aquaculture ponds (Liu et al., 2013), whereas it turned to WT, depth, and phytoplankton abundance during the cloudy days. This finding is consistent with a previous work in intensive aquaculture ponds, indicating that the vertical distribution of DO was closely related to weather conditions. A rainstorm or sudden change in wind direction can cause a mixing of DO in different water layers (Ryabov et al., 2010). Previous work showed that when the concentration of DO < 3 mg/L, the food intake of shrimp decreased significantly. When the dissolved oxygen content drops below 2.0 mg/L, shrimp hardly feed (Wu et al., 2002).

The vertical distribution of phytoplankton abundance would directly affect the vertical distribution of Chl-a in aquaculture ponds (Sriyasak et al., 2013) and indirectly affect the vertical distribution of DO. The photosynthesis of phytoplankton to produce DO mainly occurs during the daytime, and phytoplankton consume DO and lead to short-term hypoxic conditions during the nighttime (Culberson and Piedrahita, 1996). The dominant phytoplankton species in aquaculture ponds are cyanobacteria and green algae (Lukwambe et al., 2015; Cires and Ballot 2016; Rao et al., 2021). Previous works have shown that the dominance of cyanobacteria is highly related to TP concentration (Ho et al., 2019). When the TP concentration is close to 0.1 mg/L, the probability of cyanobacterial blooms can reach 80% (Downing et al., 2001). In this study, the concentration of TP in all aquaculture ponds exceeded 0.1 mg/L (**Table 1**), which resulted in a high phytoplankton abundance and indirectly affected the concentration of DO in the water column.

## Management implications in aquaculture ponds

Concentrations of DO lower than 1.0 mg/L will result in hypoxic conditions in aquaculture ponds, and the tolerance of large fish to hypoxia is higher than that of small fish (Wang et al., 2017). Currently, aquaculture ponds are threatened by hypoxic conditions due to climate change and human activity, which result in a large number of fish deaths (Chang, 1986). A long period of low DO in aquaculture ponds will change the chemical conditions, for instance, the increase in nitrite, ammonia, and hydrogen sulfidic concentrations, and finally lead to the eutrophication of fish ponds. The deterioration of water quality will increase fish deaths and cause large economic losses (Conley et al., 2009).

#### Conclusion

Our study highlights the impact of thermal stratification and mixing on the vertical distribution of DO in aquaculture ponds. Apparent vertical distributions of DO, Chl-a, and phytoplankton abundance were observed during the daytime, with concentrations decreasing with increasing water depth. Linear regression models showed that WT, Chl-a, and phytoplankton abundance significantly impacted the vertical distribution of DO. Given that hypoxic conditions are likely to be more frequent under future climate change and human activity, our findings provide important insights into the management of aquaculture ponds.

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