

# A PRELIMINARY STUDY ON ECOLOGICAL SUITABILITY EVALUATION BENCHMARKS FOR DISSOLVED OXYGEN (DO) IN GRASS CARP AQUACULTURE PONDS

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(Received 7<sup>th</sup> Aug 2025; accepted 10<sup>th</sup> Nov 2025)

**Abstract.** Dissolved oxygen (DO) is a critical environmental factor determining the survival, health, and growth efficiency of grass carp (*Ctenopharyngodon idella*), which is the most productive freshwater aquaculture species in China. However, the lack of standardized, practical benchmarks for DO suitability has hindered effective water quality management in grass carp ponds. This study aims to establish a set of ecologically relevant DO benchmarks for grass carp through a systematic synthesis of published physiological and ecological research. We conducted a comprehensive, literature-based synthesis by retrieving studies from major databases including Web of Science, Scopus, and the China National Knowledge Infrastructure (CNKI). Data on DO concentrations and corresponding physiological or ecological responses of grass carp were extracted, compared, and analyzed to define distinct suitability levels. The analysis resulted in five ecological suitability levels with the following DO benchmarks (ranges): death period ( $\leq 0.60$  mg/L), danger period (0.60–1.60 mg/L), impact period (1.60–4.70 mg/L), normal period (4.70–7.50 mg/L), and optimal period ( $\geq 7.50$  mg/L). For reliable monitoring, it is recommended that water samples be collected at dawn from near the pond bottom in both upwind and downwind areas, with the lowest measured DO value used for assessment. The proposed benchmarks offer a scientifically grounded and practical tool for aquaculture practitioners to diagnose DO status and implement timely interventions, thereby promoting healthy cultivation and sustainable production of grass carp.

**Keywords:** *grass carp (Ctenopharyngodon idella), dissolved oxygen, ecological suitability, benchmark, aquaculture management, meta-analysis, hypoxia, water quality, physiological response*

## Introduction

Dissolved oxygen (DO) is a critical parameter in assessing water quality within aquaculture systems. A substantial body of research indicates that DO concentration not only directly impacts fish survival, growth, disease resistance, and overall production efficiency (Xu et al., 2022) but also influences indirectly the transformation and accumulation of other chemical substances in the aquatic environment (Kang, 2012). Under conditions of sufficient DO, fish exhibit optimal physiological performance, including high feed conversion rates. Conversely, when DO levels are inadequate, the decomposition of organic matter may proceed anaerobically, resulting in the generation of toxic byproducts such as hydrogen sulfide and ammonia. These conditions are often accompanied by a decline in pH and an increase in carbon dioxide (CO<sub>2</sub>) concentration, contributing to a phenomenon known as biological hypoxia. Biological hypoxia can lead to elevated CO<sub>2</sub> levels in the blood and a corresponding decrease in blood pH, which ultimately impairs the blood's capacity to transport oxygen. If water quality is not promptly restored, this scenario may trigger a self-reinforcing cycle that intensifies hypoxic conditions and may ultimately result in fish mortality (Pan, 1996).

Grass carp (*Ctenopharyngodon idella*) is a member of the family Cyprinidae, subfamily Leuciscinae, and genus *Ctenopharyngodon*. At present, grass carp ranks first in aquaculture production among the Four Major Farmed Carps in China, and is widely distributed across various farming regions, demonstrating significant potential for further development. Experimental studies have demonstrated that the growth rate of grass carp at a dissolved oxygen (DO) concentration of 2.7 mg/L is 9.88 times lower than that at 5.56 mg/L, with the feed conversion ratio reaching up to four times higher (Yang, 1997). When DO levels are 3.5 mg/L compared to 6.0 mg/L, there is a significant reduction in the apparent digestibility of protein, energy, and dry matter in the diet, along with a decrease in food intake. Moreover, key growth and nutritional performance indicators, including weight gain, protein retention rate, protein efficiency, feed conversion rate, and amino acid retention rate—exhibit notable declines (Gan et al., 2013). The concentration of dissolved oxygen in water is undoubtedly one of the most critical environmental factors influencing the healthy growth and survival of grass carp (Xu et al., 2022).

Many aquaculture farmers generally pay more attention to the phenomenon of fish floating at the surface or dying due to severe oxygen deficiency. However, they often overlook or fail to fully understand the implications of DO concentrations that do not immediately threaten the survival of grass carp but may still significantly affect their physiological performance. A considerable number of domestic and international studies have already explored the effects of DO on the physiological ecology of grass carp. Nevertheless, discrepancies among research findings are common, largely due to variations in experimental conditions such as the body weight of test subjects, methodologies employed, and targeted DO levels—particularly in terms of determining ecologically suitable DO thresholds for optimal grass carp performance. For most small-scale fish farmers in China, effectively applying these scientific findings to accurately and promptly assess appropriate DO levels in aquaculture ponds remains a significant challenge, thereby hindering the implementation of timely and evidence-based management strategies.

This paper presents a literature-based synthesis that proposes a set of evaluation benchmarks for determining the ecological suitability of DO levels in grass carp farming ponds. The main research aim is to establish a set of DO ecological suitability benchmarks for grass carp aquaculture ponds, based on a systematic compilation and analysis of existing physiological and ecological studies. We hope that these benchmarks can serve as a helpful reference for aquaculture farmers around the world in managing pond DO levels safely and adjusting ecological conditions, ultimately supporting the healthy and sustainable growth of grass carp.

## Materials and methods

Using databases such as Web of Science, Scopus, China National Knowledge Infrastructure - Chinese Journal Full-text Database, and China Doctoral and Master's Theses Full-text Database, full-text searches were conducted using the keywords 'dissolved oxygen' in both Chinese and English, 'grass carp' in both Chinese and English, and its Latin name '*Ctenopharyngodon idella*'. Studies were included if they reported DO concentrations and corresponding physiological or ecological responses in grass carp. Exclusion criteria included studies not focusing on *C. idella*, those without DO measurements, or reviews without original data. Each study examining the effects of DO on the physiological ecology of grass carp was carefully selected. Based on these

studies and their referenced literature, data were systematically analyzed and organized in ascending order of DO levels to illustrate the key physiological and ecological responses of grass carp under varying DO conditions. This dataset provides a scientific foundation for establishing ecological suitability evaluation benchmarks. This article does not classify ecological suitability using traditional categories such as high, medium, low, or excellent, good, moderate, poor. Instead, to improve clarity and practical application for fish farmers, DO suitability for grass carp growth and survival is divided into five levels: optimal period, normal period, impact period, danger period, and death period. Each level has a corresponding evaluation criterion—referred to as the optimal, normal, impact, danger, and death period benchmarks. The definitions of these levels are as follows:

(1) Optimal period: When grass carp are at the theoretically most ideal DO concentration, all routine physiological indicators are not affected by DO stress.

(2) Normal period: The period from the optimal state to when detectable adverse effects (such as significantly slowed growth) begin to appear.

(3) Impact period: The period from when adverse effects (including physiological indicators) start to appear until the threat of death (such as surfacing, loss of balance) begins to appear.

(4) Danger period: The period from when grass carp start facing a threat of death to when individual fish begin to die within 24 h.

(5) Death period: The period after grass carp start dying until it may lead to more individuals dying.

The DO threshold for each ecological suitability level is determined by identifying consistent response points across multiple studies, prioritizing findings supported by repeated observations or multiple sources, and considering various factors such as experimental subjects and conditions.

## Results

### *Summary of research on the effects of DO on the physiological ecology of grass carp*

This study collected over 10 research papers related directly to DO and grass carp from both domestic and international sources. The lowest recorded DO concentration was 0.30 mg/L (Ye, 1959), while the highest was 8 mg/L (Zhu, 2004). The earliest research paper was published in 1955 (Chen and Shi, 1955), and the latest in 2023 (Wu et al., 2023). The recorded initial weight of the study individuals ranged from as low as 0.72 g (Ye, 1959) to as high as an average of 327 g (Zhao et al., 2018). The physiological and ecological responses of grass carp under different DO concentrations are detailed in *Table 1*.

**Table 1.** Summary of research results on the physiological and ecological effects of grass carp under different DO concentrations (sorted from low to high DO concentration)

Body wet weight (g)	Body length (cm)	DO (mg·L <sup>-1</sup> )	Physiological and ecological responses	Sources
0.72	3.3	0.30-0.51	All eventually suffocated to death	Ye (1959)
10-12		0.38	The critical oxygen tension at which the body loses balance (10°C)	Xu et al. (2022)
-	12.6 (full length)	< 0.39	Dead individuals have been found	Chen and Shi (1955)
175.6 ± 18.8	-	0.39 ± 0.003	The critical point of asphyxiation death	Yu et al. (2017)
-	-	0.5	The critical point of asphyxiation death	Zhu (2004)

327 ± 66		0.63	The respiratory stroke volume and gill ventilation reach their maximum values (25°C)	Zhao et al. (2018)
10-12		0.64	The critical oxygen tension at which the body loses balance (25°C)	Xu et al. (2022)
214.6 ± 18.7	24.06 ± 0.53	0.65 ± 0.05	The mortality has reached 50%	Wu et al. (2023)
0.72	3.3	0.68	Mass fish surfacing and gasping observed	Ye (1959)
7.6-14.6	-	0.70	The critical oxygen tension. Below this concentration, sufficient oxygen cannot be obtained to maintain its basal metabolism, forcing it into a state of metabolic inhibition, facing survival threats (30°C)	Chen et al. (2019)
327 ± 66	-	0.72	The respiratory stroke volume and gill ventilation reach their maximum values, while the respiratory frequency also reaches its peak (30°C)	Zhao et al. (2018)
10-12		0.79	The critical oxygen tension at which the body loses balance (33°C)	Xu et al. (2022)
-	13.5 (full length)	< 0.99	Unconscious and incapacitated	Chen and Shi (1955)
-	-	1.0	Re-adjust the fish gill organ	Xu et al. (2019)
17.4 ± 0.39	9.8 ± 0.07	1.0	No deaths observed within 48 h	Zhang et al. (2013)
327 ± 66	-	1.09	Gill ventilation and respiratory stroke volume reach their maximum values (15°C)	Zhao et al. (2018)
0.72	3.3	1.44	The phenomenon of fish surfacing and gasping began to be discovered	Ye (1959)
327 ± 66		1.48	The respiratory frequency has reached its maximum (25°C)	Zhao et al. (2018)
-	-	1.6	The phenomenon of fish surfacing and gasping began to be discovered.	Zhu (2004)
327 ± 66		1.78	Critical oxygen tension for oxygen extraction efficiency (30°C)	Zhao et al. (2018)
327 ± 66		1.82	Critical oxygen tension for oxygen extraction efficiency (25°C)	Zhao et al. (2018)
327 ± 66	-	1.84	Critical oxygen tension for oxygen consumption rate (30°C)	Zhao et al. (2018)
327 ± 66	-	2.02	Critical oxygen tension for oxygen consumption rate (25°C)	Zhao et al. (2018)
327 ± 66		2.09	Respiratory frequency reaches maximum (15°C)	Zhao et al. (2018))
		2.22	Critical oxygen tension for oxygen extraction efficiency (15°C)	
327 ± 66		2.42	Critical oxygen tension for oxygen consumption rate (15°C)	Zhao et al. (2018)
327 ± 66	-	<2.54	Oxygen consumption rate and oxygen extraction efficiency have significantly decreased, while respiratory frequency and respiratory stroke volume began to significantly increase (30°C)	Zhao et al. (2018)
214.6 ± 18.7	24.06 ± 0.53	2.57 ± 0.33	Survival rate of 100%, but low feeding rate and slow growth (factory-scale recirculating water aquaculture)	Wu et al. (2023)
327 ± 66	-	2.91	Oxygen consumption rate began to significantly decrease; respiratory frequency and respiratory stroke volume began to significantly increase (25°C)	Zhao et al. (2018)
327 ± 66	-	<3.09	The oxygen consumption rate begins to significantly decrease (15°C)	Zhao et al. (2018)
4.11 ± 0.03		3.50	Compared to the group with a concentration of 6.0 mg/L, its food intake and apparent digestibility of protein, energy, and dry matter were significantly reduced; it was harmful to the liver	Gan et al. (2013)
327 ± 66	-	<3.66	Gill ventilation begins to significantly increase (30°C).	Zhao et al. (2018)
327 ± 66	-	<3.92	Gill ventilation begins to significantly increase (25°C)	Zhao et al. (2018))
214.6 ± 18.7	24.06 ± 0.53	4.61 ± 0.11	Survival rate of 100%, unaffected growth compared to the group exposed to a concentration of 6.50 ± 0.48 mg/L (industrialized recirculating water aquaculture)	Wu et al. (2023)
100.1-102.3		4.66-7.89	Demonstrating normal growth rates farmed in ponds	Wang et al. (2017)
	-	5-7	Normal growth with low motor ability	Pan (1996)
327 ± 66	-	<5.44	Respiratory frequency and respiratory stroke volume began to significantly increase at 15°C	Zhao et al. (2018)
-	-	5-8	Appear excellent growth rates	Zhu (2004)
214.6 ± 18.7	24.06 ± 0.53	6.50 ± 0.48	Survival rate of 100%, with no significant difference in growth compared to the group maintained at a concentration of 4.61 ± 0.11 mg/L (industrialized recirculating water aquaculture)	Wu et al. (2023)

- indicates that the original literature did not provide relevant data. The number of significant digits is the same as in the original literature. All temperature values refer to water temperature

### ***The evaluation benchmark for ecological suitability***

The boundary values were determined by integrating consistent findings from multiple studies, with a conservative approach applied to ensure practical safety margins.

#### *Danger and death period benchmarks*

Low levels of DO can threaten the survival of grass carp, even leading to death. Studies have shown that juvenile grass carp weighing between 7.6-14.6 grams, under water temperatures of 30°C, reach critical oxygen tension at a DO concentration of 0.70 mg/L. This indicates that below this concentration, grass carp cannot obtain sufficient oxygen to maintain their basic metabolism and are forced into a state of metabolic inhibition, facing survival threats (Chen et al., 2019). Grass carp measuring 13.5 cm in total length exhibited severe surfacing and gasping behavior when the DO concentration dropped to 0.68 mg/L (Ye et al., 1996). Grass carp weighing 10-12 grams, under water temperatures of 25°C, began to lose balance when the DO concentration fell to 0.64 mg/L (Xu et al., 2022). On average, grass carp weighing  $327 \pm 66$  grams, under water temperatures of 25°C, reached maximum values for respiratory stroke volume and gill ventilation at a DO concentration of 0.63 mg/L, but no deaths were observed during the experiment (Zhao et al., 2018).

The above results indicate that when dissolved oxygen (DO) concentration drops to 0.63 mg/L, it threatens the survival of grass carp but has not yet reached the lethal level. Studies have shown that the lethal DO concentration for grass carp is 0.5 mg/L (Zhu, 2004); for fish weighing  $175.6 \pm 18.8$  g, the lethal level is  $0.39 \pm 0.003$  mg/L (Yu et al., 2017); those measuring 12.6 cm in length died when DO fell below 0.39 mg/L (Chen and Shi, 1955); and 0.72 g grass carp died when DO was between 0.30–0.51 mg/L (Ye et al., 1996). These findings suggest that DO levels below 0.5 mg/L can lead to significant mortality. Considering that fish tolerance to low DO decreases at higher water temperatures, this study adopts a precautionary approach and sets the death period threshold at  $\leq 0.60$  mg/L.

Research shows that grass carp weighing  $327 \pm 66$  g have a critical oxygen requirement (the minimum oxygen concentration that does not affect respiration) of 1.84 mg/L at 30°C (Zhao et al., 2018). Fish begin to surface and gasp when DO drops to 1.6 mg/L (Zhu, 2004). Grass carp fry (average length 3.3 cm, weight 0.72 g) shows similar behavior at 1.44 mg/L under water temperature 29.0–29.5°C, pH 6.9–7.0, and CO<sub>2</sub> levels of 27.85–30.27 mg/L (Ye et al., 1996). A 13.5 cm grass carp became unconscious at 0.99 mg/L under 24°C, pH 7.1, and CO<sub>2</sub> of 10.4 mg/L (Chen and Shi, 1955), indicating dangerous DO levels. In another study, fish weighing  $17.4 \pm 0.39$  g and measuring  $9.8 \pm 0.07$  cm in length survived for 48 h at a stable DO level of 1.0 mg/L (Zhang et al., 2013).

Based on the above research, grass carp begin to surface when DO levels are between 1.44 and 1.60 mg/L. To provide an early warning for real-world farming conditions, this study sets the danger period threshold at 1.60 mg/L, with the danger period range defined as 0.60 to 1.60 mg/L.

#### *The benchmarks for impact period, normal period and optimal period*

Studies show that at 25°C water temperature, grass carp begin to show a significant increase in gill ventilation when DO drops to 3.92 mg/L (Zhao et al., 2018), indicating that DO below this level may affect normal growth. Data also show that grass carp with

an initial weight of  $214.6 \pm 18.7$  g have a 100% survival rate and maintain normal growth at  $4.61 \pm 0.11$  mg/L DO, with no significant difference compared to groups at  $6.50 \pm 0.48$  mg/L (Wu et al., 2023). Fish weighing 100.1–102.3 g also grow well at DO levels between 4.66 and 7.89 mg/L (Wang et al., 2017). Some researchers suggest that suitable DO levels range from 5 to 8 mg/L (Zhu, 2004). According to China's "Fishery Water Quality Standards," DO in fishpond should remain above 5 mg/L for at least 16 h daily and never fall below 3 mg/L otherwise. Based on these findings, this study defines the impact period DO range for grass carp as 1.60–4.70 mg/L, with 4.70 mg/L being the minimum for normal growth.

Some researchers suggest that for high yields in fish fry breeding ponds, DO should be above 7 mg/L. Levels below 5 mg/L are not ideal for productivity, and below 3 mg/L can harm fish and other aquatic organisms (Pan, 1996). In some ponds, DO may remain below the optimal range (5–7 mg/L for cyprinid fish) for long periods. While fish can still survive and grow, their activity, feeding, and immunity decline, leading to higher disease risk and lower yields. Swingle (1969) noted that DO below 1 mg/L is lethal for warm-water fish within hours, while levels above 5 mg/L support normal growth and reproduction (Wang, 1984). Based on findings from Wang et al. (2017) and Wu et al. (2023), this study defines the optimal DO concentration for grass carp as greater than 7.50 mg/L, with the normal range set at 4.70–7.50 mg/L.

It should be noted that the collected studies varied in experimental conditions, including fish size, temperature, and measurement methods. These factors may influence the precise DO thresholds and should be considered when applying the benchmarks in specific farming contexts.

### ***Examples of application***

Zhang et al. (2020) monitored average DO levels in three types of grass carp ponds in the Poyang Lake area from April to November: small-scale mountainous ponds at 7.07 mg/L, intensive ponds at 5.42 mg/L, and aquaponic ponds at 6.05 mg/L. According to this study's ecological suitability benchmarks, all values fall within the normal period. Tang et al. (2013) measured DO in intensive grass carp polyculture ponds (with black carp, crucian carp, silver carp, and bighead carp) in Huzhou City from May to July 2009, averaging 3.84–5.93 mg/L—between the impact and normal periods. From August to November, DO dropped to 2.02–2.29 mg/L, entering the impact period and nearing the danger period. Prolonged exposure to such low DO can severely affect grass carp growth and survival. The authors indeed reported frequent fish deaths, consistent with this study's evaluation. Li et al. (2014) monitored DO in grass carp ponds in Hubei Province from May to October 2012 (with silver carp, bighead carp, and mixed feed), recording a maximum of 12.63 mg/L and minimum of 2.63 mg/L, corresponding to the optimal and impact periods, respectively.

### **Discussion and conclusion**

In aquaculture ponds, DO concentrations vary over time and space, showing horizontal and vertical differences as well as daily fluctuations. Wind direction and speed mainly determine the horizontal oxygen distribution. Without wind, oxygen is evenly distributed. With wind, surface water rich in oxygen and phytoplankton moves to the downwind shore, while oxygen-poor bottom water rises on the upwind side. During the day, phytoplankton photosynthesis further increases DO by 1–2 mg/L or more in the

downwind area. At night, the same phytoplankton consume oxygen through respiration, lowering DO levels (Pan, 1996; Ruan and Yang, 2006).

The vertical DO distribution in aquaculture ponds is influenced by weather. On sunny days, phytoplankton photosynthesis causes oxygen levels to decrease with depth, while oxygen consumption remains relatively stable across water layers. As a result, DO generally declines from the surface to the bottom (Pan, 1996; Ruan and Yang, 2006; Gu et al., 2009; Gong et al., 2013). On rainy or cool days, surface water cools and sinks, mixing with deeper layers and equalizing DO levels (Guan, 2004). From dusk to midnight, DO steadily drops, and vertical differences become less pronounced (Pan, 1996). Studies of grass carp ponds along the Yellow River show that between May and October, DO near the bottom (about 10 cm above) is significantly lower than in upper layers (Li et al., 2001). In 1.5–2.0 m deep, high-density grass carp ponds, photosynthetic oxygen production decreases with depth, creating an oxygen deficit at the bottom. Oxygen consumption remains similar across layers. Surface water produces more oxygen than it consumes, while middle and bottom waters consume more than they produce (Gong, 2013). These findings indicate that low-oxygen zones commonly form at the bottom of grass carp ponds.

Daily DO levels in aquaculture ponds fluctuate due to respiration, photosynthesis, and slow oxygen diffusion. During the day, algae produce oxygen through photosynthesis, while at night, oxygen is only consumed, leading to a pattern of increasing DO after sunrise, peaking before sunset, and declining overnight, reaching its lowest near dawn (Wang, 1984; Ruan and Yang, 2006; Zhang et al., 1984). Studies show that from April to November, DO at 6:00 AM is significantly lower than at 12:00 PM and 6:00 PM, with the lowest levels recorded at 1.48 mg/L in June and 1.70 mg/L in May (Li et al., 2001).

To ensure fishery production safety, ecological suitability assessments of aquaculture ponds should use the lowest daily DO value, following a conservative approach. Therefore, DO monitoring should be conducted at dawn, with samples collected near the pond bottom from both upwind and downwind areas. The lowest DO value should be used for evaluation. Furthermore, the largest grass carp studied in current DO physiology research weighs only  $327 \pm 66$  grams. However, in commercial ponds, grass carp typically grow larger than this. Current research does not clarify whether size significantly affects DO tolerance. Therefore, future studies should focus on testing and collecting field data on larger grass carp.

This study provides a systematically derived set of DO suitability benchmarks for grass carp aquaculture, offering a practical tool for water quality management. Based on the above research and analysis, this paper divides the ecological suitability of DO in grass carp ponds into five levels from least to most suitable: death period, danger period, impact period, normal period, and optimal period. Domestic and international studies on DO's effects on grass carp physiology were systematically reviewed. Using comparative analysis and following a cautious approach, the DO thresholds for each level were set as follows: death period  $\leq 0.60$  mg/L, danger period 0.60–1.60 mg/L, impact period 1.60–4.70 mg/L, normal period 4.70–7.50 mg/L, and optimal period  $>7.50$  mg/L. Future research should focus on validating these thresholds in commercial-scale ponds and for larger grass carp, which are underrepresented in current physiological studies. DO monitoring should be conducted near the pond bottom before dawn, in both upwind and downwind areas, either in situ or through water sampling. For fish farmers, if DO falls into the danger or death period, immediate oxygen-increasing measures are needed to prevent fish loss. If DO is in the impact period, close attention to weather changes is advised to avoid further decline.

**Conflict of interest:** The authors declare that they have no conflict of interest.

**Acknowledgements.** The authors would like to express gratitude for the financial support from the National Natural Science Foundation of China (Grant No.: 52270179).

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