Ecological determinants of yellow flesh discoloration in *Pangasianodon hypophthalmus* (Sauvage, 1878): a case study in Deli Serdang Regency, North Sumatra, Indonesia

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Abstract. Aquaculture sector cultivating giant catfish (*Pangasionodon hypophthalmus*) in North Sumatra currently grapples with a diminishing economic outlook attributed to a perplexing case of flesh discoloration. The issue surfaced in March 2022, inducing a yellow hue in the harvested fillets, with the underlying cause remaining elusive. This discoloration, a result of intricate interactions between abiotic factors (environmental conditions) and biotic factors (organisms), highlights the complexity of aquaculture challenges. This study aimed to unravel the ecological interplay between these abiotic and biotic elements within the pond environment, specifically in connection to the yellow flesh discoloration observed in catfish. To conduct a comprehensive analysis, we established fish size clusters as sample units, categorized according to the day of culture (DOC): cluster-A (DOC ≤ 100), cluster-B (100 ≤ DOC ≤ 200), and cluster-C (DOC ≥ 200). Each cluster was observed three times over a two-week period. Observations included an assessment of various fish conditions, such as the degree of yellowness, evaluated using the CIELAB classification (L for brightness, a* for redness, and b* for yellowness), and total flesh carotenoid levels. The environmental context was simultaneously examined, with a focus on key physical-chemical factors, including water temperature, pH, redox potential, total dissolved solids, oxygen solubility, ammonia, nitrate, nitrite, total carotenoids in water, and ecological parameters of plankton. The intricate influence of size clusters and observation periods on both abiotic and biotic factors was highlighted by our findings. Adverse water conditions - low oxygen solubility, elevated total dissolved solids, and excessive levels of ammonia, nitrate, and nitrite - were noted, surpassing regulatory thresholds. An increase in L and b* values, coupled with a decline in a* values towards the conclusion of the observation period, indicated an impending yellow flesh discoloration in all patin catfish. Furthermore, a discernible linear correlation was observed between water-sourced carotenoids and flesh carotenoid content. The phytoplankton group, primarily Cyanophyceae, displayed higher taxa and abundance compared to zooplankton, emphasizing the intricacies of the ecosystem. Our findings were consolidated through multivariate analysis using Principal Component Analysis (PCA), highlighting a robust correlation between carotenoid variations and the presence of *Anabaena* sp. and *Phormidium* sp. in the aquaculture pond. In navigating the complexities of giant catfish aquaculture, our study advocates for holistic approaches that balance environmental sustainability with economic viability.

Key Words: *Anabaena,* CIELAB, carotenoid, multivariate, *Phormidium*, Principal Component Analysis (PCA).

Introduction. Indonesia possesses a wealth of fisheries resources, encompassing marine, freshwater, and brackish water fisheries. With a vast water area reaching 6.4 million km^2, Indonesia has the potential to become the world's largest fisheries producer. Fisheries production in Indonesia comprises both capture fisheries and aquaculture. Major aquaculture commodities include milkfish (*Chanos chanos*), tilapia (*Oreochromis niloticus*), catfish (*Clarias* spp.), catla (*Catla catla*), carp (*Cyprinus carpio*), and gourami (*Osphronemus goramy*). One promising aquaculture commodity for development is catfish, particularly due to its high economic value (KKP 2022). Catfish species commonly cultured in tropical waters, holding potential as a superior aquaculture commodity in Indonesia, belong to the giant catfish or *Pangasius* spp. group (Ferraris 2007). Based on data regarding annual productivity of cultured catfish in Indonesia from 2010 to 2021, there has been an average production increase of 11.02%. However, this figure still falls significantly short of annual targets, reaching only 66% in 2022. Meanwhile, catfish production rates in North Sumatra tend to experience an average increase of 4.83% (KKP 2022). The notable rise in catfish production in North Sumatra suggests the region holds potential and a prospective market share for catfish aquaculture endeavors. Flesh discoloration is defined as a change in color and condition from the initial state, indicating a decrease in quality. Based on local reports, this case has been occurring since March 2022 and has affected giant catfish ponds in Deli Serdang Regency, North Sumatra, Indonesia. The repercussions of this case may extend to the local and regional economic sectors, given the high demand for this commodity and the threat of decreased purchasing power by the community due to the ongoing issue.

Cases of catfish flesh discoloration have been reported in Bangladesh, occurring in the Tra catfish species, *Pangasianodon hypophthalmus* (Hoque et al 2021). Consumers generally prefer white fish meat, unless specific species with particular colors, such as the pink flesh of salmon, are well-established (Altintzoglou et al 2022). Yellow meat signifies low quality, impacting sales both domestically and in export markets like Russia, Vietnam, and various European countries. While discoloration hampers catfish exports from Bangladesh, other Asian countries like Vietnam consistently export white-fleshed fish to Europe and other international markets (Belton et al 2011). The phenomenon of discoloration arises from a combination of supportive abiotic (environmental) factors and abundant biotic factors (such as phytoplankton) in a spatiotemporal manner, depending on the cultivation conditions. The yellow color in catfish meat is typically caused by yellow-orange carotenoid pigments, such as carotene and its oxygenated derivatives, known as xanthophylls. Various fish species accumulate different types of carotenoids, but xanthophylls, lutein, and zeaxanthin are considered the main yellow pigments in catfish (Li et al 2007).

Carotenoids are synthesized by microorganisms and plants and are not produced by animals (Islam et al 2021). Therefore, catfish carotenoids are derived either from external sources or the environmental habitat (cultivation). In commercial production, carotenoids can be included in feed or come from materials or microorganisms consumed by fish in the water. Catfish are omnivorous and opportunistic organisms that, in their natural habitat, feed on algae, aquatic plants, crustaceans, and other organisms in the water (Zimba et al 2003). This suggests that the presence of natural organisms containing carotenoids in the water in fish ponds contributes to the accumulation of carotenoids in catfish meat, both accidentally and incidentally. Hu et al (2013) reported a correlation between xanthophyll content and phytoplankton abundance (microorganisms) as a biotic determinant of yellow flesh discoloration in channel catfish (*Ictalurus punctatus*) in cultivation ponds. Da et al (2016) added that biotic factors such as phytoplankton communities, including Chlorophyceae (59.4%), Bacillariophyceae (17.4%), Euglenophyceae (12.2%), and Cyanophyceae (11.4%), combined with abiotic factors such as low oxygen levels and high ammonia levels, likely contribute to incidents of discoloration in fish. Other researchers affirmed that environmental conditions such as low dissolved oxygen levels and high ammonia (NH_3) content are the causes of discoloration in catfish (Qiufen et al 2012). Based on the theoretical framework and presented conditions, this research is focused on several questions related to the case of flesh discoloration in Deli Serdang Regency. Firstly, what is the environmental condition or the physical-chemical factors of water as the initial basis for assessing the quality of giant catfish cultivation? Secondly, is there a sufficiently high content of carotenoids in the water body that can influence the carotenoid levels, degree of yellowness, or even trigger cases of discoloration in catfish fillets across size clusters? Lastly, is there an identifiable relationship between the environmental conditions in the catfish cultivation pond, including the abundance and community of phytoplankton, and other abiotic factors, with the ongoing case of discoloration? By detailing these questions, the study aimed to provide a deeper understanding of the factors influencing the case of catfish flesh discoloration in the fisheries cultivation environment.

Material and Method

Experimental design. The method employed in this research includes survey, observation, and data collection techniques, involving the gathering of primary data based on the observed variables within the catfish cultivation units. This study is ex post facto, presenting initial information in the form of a systematic, factual, and accurate overview based on field facts and the relationships among investigated phenomena. The study scope encompassed the quality and external factors in one of the giant catfish cultivation ponds in Deli Serdang Regency, North Sumatra, Indonesia. The data were collected from August to October 2023.

Sampling population and size. A total of three experimental catfish ponds, selected purposively among cultivation ponds in each catfish group based on the day of culture (DOC) classification - cluster-A (DOC ≤ 100 days), cluster-B (100 ≤ DOC ≤ 200), and cluster-C (DOC ≥ 200) (N = 15), were chosen as replicates. Water samples and catfish specimens were collected every 2 (two) weeks. The first week was designated as the initial sampling period (I), continuing until the third or final sampling period (III), with a total observation period of six weeks.

Water quality parameters. In this study, water samples from the catfish cultivation ponds were measured for physical-chemical quality factors based on temperature (°C) using a digital thermometer, dissolved oxygen (DO) content using a DO-meter (mg L^{-1}), water acidity level (pH) using a digital pH-meter, redox potential (EH), total dissolved solids (TDS), ammonia, nitrate, and nitrite levels (mg L^{-1}) adjusted to SNI 06-6989.9-2004 standards. Water sampling is conducted between 09:00 to 10:00 AM every 2 weeks over a 2-month observation period in three cultivation ponds.

Phytoplankton sampling. The abundance and composition of phytoplankton species in the catfish pond were determined by collecting water samples. The water samples were initially disinfected using a 70% detergent/alcohol solution to eliminate adhering microorganisms. Samples were taken at each location from 4 different points and then combined into one. The samples were collected approximately 10 cm below the water surface. A 5 L bucket was used for sample collection, filtered through a plankton net six times to achieve a total sampling volume of 30 L. The filtered results were stored in sample bottles, each containing 100 mL, and preserved with 0.3 mL of Lugol's iodine as a preservative (Suhenda 2009).

Catfish sampling. Fish samples from the catfish pond were randomly collected (n = 5) from each pond every 2 weeks over three observation periods. The fish samples were captured using a scoop net, and the muscle (flesh) condition was visually examined to indicate the progression of abnormalities from the beginning to the end of the study. Visual symptoms included changes in muscle color, with 10 randomly selected observation areas sampled using the tool at https://trigit.com.au/ to obtain brightness/lightness (L), redness degree (a*), and yellowness degree (b*) values (Tjandra et al 2023). Muscle fillet samples were collected and stored under cold conditions (4°C) for measuring carotenoid levels.

Data analysis. The results of water quality testing were presented as the mean and standard deviation from three designated experimental ponds. Statistical analysis was applied to the same parameter groups at different observation intervals using One-way ANOVA in Minitab ver. 19.0. Post-hoc tests were conducted in case of significant differences ($p \leq 0.05$) using the Tukey's test to determine statistically similar or different test groups. Multivariate analysis, specifically Principal Component Analysis (PCA), was employed to establish connections between physicochemical factors of water and biotic factors (plankton communities and carotenoid profiles) in Origin ver. 2023b. Plankton identification was conducted using a binocular microscope with a magnification of 100x. Water samples in bottles were extracted using a pipette and placed on a glass slide for immediate observation under the microscope. Phytoplankton density calculations were performed using a specialized Sedgwick-Rafter counting chamber in cells m^{-3}, along with other ecological indices such as Shannon's diversity index, Simpson's dominance index, and Pielou's evenness index. Total carotenoid content in catfish muscle tissue and water samples (plankton) was measured using colorimetry on a UV-Vis spectrophotometer. Tissue samples (40-50 mg) were placed in a reaction flask and mixed with 5 mL of acetone, followed by homogenization. An additional 10 mL of acetone was added and mixed until homogeneous. The filtrate was then filtered using Whatman paper and measured at wavelengths of 380, 450, 475, and 500 nm.

Results

Water quality. The water conditions in the environment of the catfish (*P. hypophthalmus*) cultivation ponds were monitored at intervals of two weeks over three observation periods. Five cultivation ponds were utilized as representative samples for each age class (A, B, C) of *P. hypophthalmus* and as replicates for observation (Table 1). Statistical analysis using ANOVA revealed several data groups with significant differences, as indicated by different letter notations for each water quality parameter. ANOVA results for water physics demonstrated significant levels ($p < 0.05$) for all parameters, including temperature ($F_{8,36} = 6.36$), pH ($F_{8,36} = 4.84$), redox potential ($F_{8,36} = 18.23$), total dissolved solids ($F_{8,36} = 111.78$), and dissolved oxygen levels ($F_{8,36} = 5.72$). Similar results were obtained for water chemistry with significant levels for ammonia content ($F_{8,36} = 90.96$), nitrite levels ($F_{8,36} = 60.49$), and nitrate levels ($F_{8,36} = 119.14$). This indicates that the conditions of the cultivation ponds undergo changes depending on the determined size classes of *P. hypophthalmus* and the duration of fish maintenance in the ponds.

Table 1

Water quality parameters in the pond of *P. hypophthalmus* in different observation periods and age groups

Parameters	*Cluster-A (Period)*			*Cluster-B (Period)*			*Cluster-C (Period)*		
	I	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
Temperature	29.36^{a}	29.40^{a}	29.60^{a}	29.54^{a}	28.80^{a}	28.80^{a}	29.56^{a}	28.50^{a}	**30.88^{b}**
(°C)	(0.49)	(0.54)	(0.54)	(0.35)	(0.83)	(0.83)	(0.43)	(0.70)	(0.54)
pH	7.18^{abc}	7.16^{abc}	7.16^{abc}	7.18^{abc}	7.12^{ab}	6.98^{a}	7.30^{bc}	**7.42^{c}**	7.38^{bc}
	(0.08)	(0.11)	(0.08)	(0.13)	(0.16)	(0.17)	(0.12)	(0.08)	(0.21)
E_H	57^{a}	64.8^{bc}	68.6^{cd}	63.6^{abc}	59^{ab}	65.8^{c}	68.6^{cd}	73.8^{de}	**75.4^{e}**
(mV)	(1.22)	(2.86)	(1.81)	(6.1)	(2.34)	(1.09)	(1.14)	(4.08)	(4.15)
TDS	826.6^{a}	837.6^{a}	971^{ab}	867^{a}	1016^{ab}	1103^{bc}	1105^{bc}	1249^{c}	**2248^{d}**
(mg L^{-1})	(16.5)	(31.8)	(40.3)	(31.9)	(46.9)	(32.4)	(157)	(46)	(209)
DO	1.05^{ab}	1.22^{bc}	**1.34^{c}**	1.06^{ab}	1.10^{ab}	1.08^{ab}	1.14^{abc}	1.07^{ab}	0.96^{a}
(mg L^{-1})	(0.04)	(0.10)	(0.08)	(0.05)	(0.10)	(0.17)	(0.15)	(0.04)	(0.05)
NH_3	0.22^{a}	3.64^{b}	5.64^{c}	0.20^{a}	6.06^{c}	6.58^{c}	3.04^{b}	5.35^{c}	**8.44^{d}**
(mg L^{-1})	(0.01)	(0.51)	(1.05)	(0.05)	(0.52)	(0.57)	(0.24)	(0.87)	(1.05)
NO_2	0.38^{a}	0.76^{a}	1.04^{a}	4.16^{bc}	3.64^{b}	**4.84^{c}**	3.54^{b}	4.20^{bc}	4.80^{c}
(mg L^{-1})	(0.02)	(0.37)	(0.32)	(0.32)	(0.51)	(0.54)	(0.58)	(0.66)	(0.83)
NO_3	0.32^{a}	2.68^{bc}	3.86^{cd}	2.36^{b}	4.40^{d}	4.88^{d}	**9.04^{f}**	7.18^{e}	7.86^{ef}
(mg L^{-1})	(0.02)	(0.43)	(0.52)	(0.21)	(0.5)	(0.63)	(0.65)	(0.68)	(0.98)

Note: DO = dissolved oxygem, E_H = redox potential, NH_3 = ammonia, NO_2 = nitrite, NO_3 = nitrate, TDS = total dissolved solids. Data represents the mean measurements from five observation ponds, with standard deviations shown in parentheses. Average values in the same row with different superscript letters indicate statistically significant differences ($p < 0.05$). Bold-printed values indicate the highest values for a specific parameter.

Visual appearances and color dynamics of fish fillets. The evaluation of yellowing or discoloration incidents in the catfish cultivation pond were observed by collecting fillet samples during each observation period. The color characteristics of *P. hypophthalmus* fillets were examined using brightness/L, redness/a*, and yellowness/b* values on the dorsal part referring to the CIELAB color space parameters. An overview of the changes in color values or characteristics in the flesh during the observation period can be seen in Figure 1. Changes in values occurred during the observation period, with varying L, a*, and b* values depending on the age groups of observed *P. hypophthalmus* (Table 2). The results indicate that the color characteristics of *P. hypophthalmus* flesh show significant outcomes in terms of brightness/L ($F_{8,81}$ = 49.05), redness/a* ($F_{8,81}$ = 36.45), and yellowness/b* ($F_{8,81}$ = 37.70) across all sampled specimens. Generally, L and b* values increased, while a* values decreased towards the end of the observation period. A significant increase in L values was observed in clusters A and C, but no significant difference was noted in cluster B. Redness or a* values did not significantly differ in cluster A but were significant in clusters B and C. Meanwhile, b* values exhibited significant differences across all catfish clusters. Ultimately, all cultivated catfish in the pond will undergo flesh discoloration, turning yellow.

Table 2

Color profile of *P. hypophthalmus* fillets based on CIELAB parameters e.g. L (brightness), a* (redness), and b* (yellowness) in different observation periods and age groups

Parameters	*Cluster-A (Period)*			*Cluster-B (Period)*			*Cluster-C (Period)*		
	I	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
L	70^{a}	76.1^{b}	77.7^{b}	84.8^{c}	86.7^{c}	86.4^{c}	87.1^{c}	88.7^{cd}	**92.4^{d}**
	(5.14)	(3.38)	(3.16)	(1.98)	(2.75)	(3.69)	(2.88)	(2.66)	(2.37)
a*	**19.3^{a}**	19.0^{a}	17.1^{a}	12.7^{b}	9.7^{b}	5.5^{c}	8.2^{b}	7.7^{b}	1.4^{e}
	(2.98)	(3.27)	(3.54)	(3.20)	(3.37)	(2.27)	(1.81)	(4.16)	(4.22)
b*	15.8^{a}	29.1^{b}	32.6^{b}	**38.5^{d}**	34.5^{c}	30.5^{b}	16.7^{a}	31.1^{b}	35.81^{c}
	(3.15)	(3.5)	(2.45)	(2.91)	(1.35)	(7.59)	(3.71)	(6.30)	(2.10)

Note: Data represents the mean measurements (N = 10) with standard deviation values shown in parentheses. Different superscript letters in the same row indicate statistically significant differences ($p < 0.05$) based on ANOVA results. Bold-printed values indicate the highest values for a specific parameter.

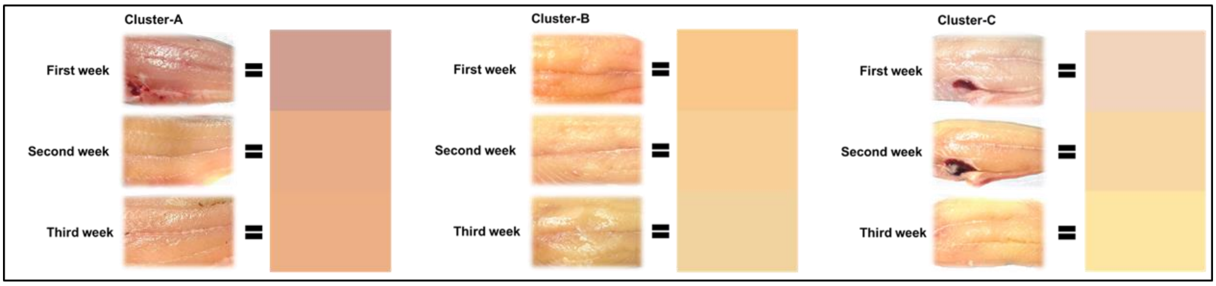

Figure 1. Color progression of *P. hypophthalmus* fillets based on age clusters and observation periods.

***Carotenoid levels and dynamics in water and P. hypophthalmus fillets**.* The color changes in catfish flesh were then examined in terms of carotenoid pigmentation, exploring the possibility of deposits originating from the cultivation pond environment or water source. Analysis results using colorimetry (spectrophotometer) technique indicate significant differences between total carotenoids in water and catfish flesh. The highest carotenoid values come from water in cluster-C, while the highest carotenoid values in flesh come from cluster-B (Table 3). Based on linear regression analysis, there is a coefficient of determination (R^2) of 0.59, indicating that the variable can contribute to data variation by 59% (Figure 2). Referring to the b* value as the yellowness parameter in catfish fillets, the R^2 values obtained are 0.70 for total carotenoids in flesh and 0.59 for total carotenoids in water (Figure 3). This suggests a relationship between the yellowness level of catfish flesh and the presence of carotenoid pigments in the cultivation environment. In general, it can be interpreted that there are specific ecological factors that contribute to the deposition of carotenoids and the observed yellow color intensity in this pond.

Table 3

Carotenoid profile of water and *P. hypophthalmus* fillets in different observation periods and age groups

Total carotenoid	*Cluster-A (Period)*			*Cluster-B (Period)*			*Cluster-C (Period)*		
	I	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
Water ($mg\ L^{-1}$)	$0.34^{a,x}$ (0.05)	$0.71^{c,x}$ (0.12)	$0.54^{b,x}$ (0.07)	$0.41^{a,x}$ (0.08)	$0.73^{c,x}$ (0.15)	$0.57^{b,x}$ (0.21)	$0.36^{a,x}$ (0.08)	**$0.87^{c,x}$** (0.21)	$0.40^{a,x}$ (0.11)
P. hypophthalmus ($mg\ kg^{-1}$)	$1.49^{b,y}$ (0.38)	$1.26^{b,y}$ (0.23)	$1.20^{b,y}$ (0.41)	$1.08^{a,y}$ (0.80)	**$2.75^{c,y}$** (0.27)	$1.82^{b,y}$ (0.24)	$1.00^{a,y}$ (0.16)	$2.60^{c,y}$ (0.22)	$0.90^{a,y}$ (0.24)

Note: Data represents the mean measurements (N = 5) with standard deviation values shown in parentheses. Different superscript letters (a, b, c) in the same row indicate statistically significant differences ($p < 0.05$) based on ANOVA results. Different superscript letters (x, y) in the same column indicate statistically significant differences ($p < 0.05$) based on t-test results. Bold-printed values indicate the highest values for a specific parameter.

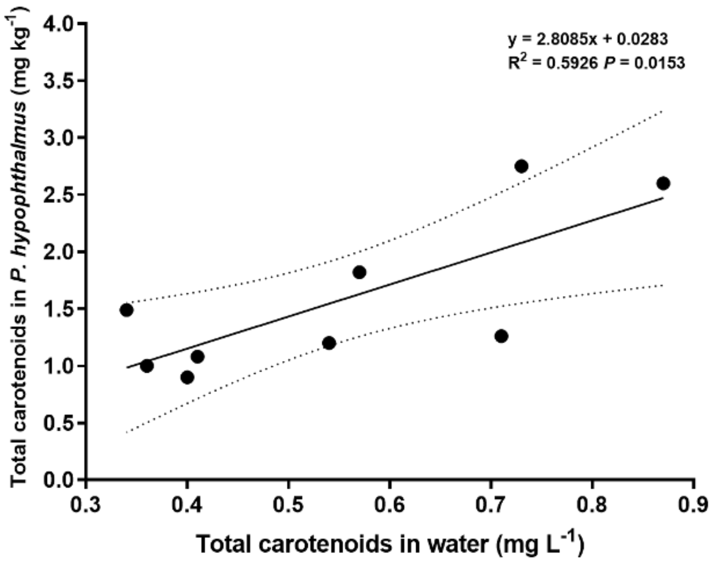

Figure 2. Linear regression curve showing the relationship between total carotenoids in water and *P. hypophthalmus* fillets.

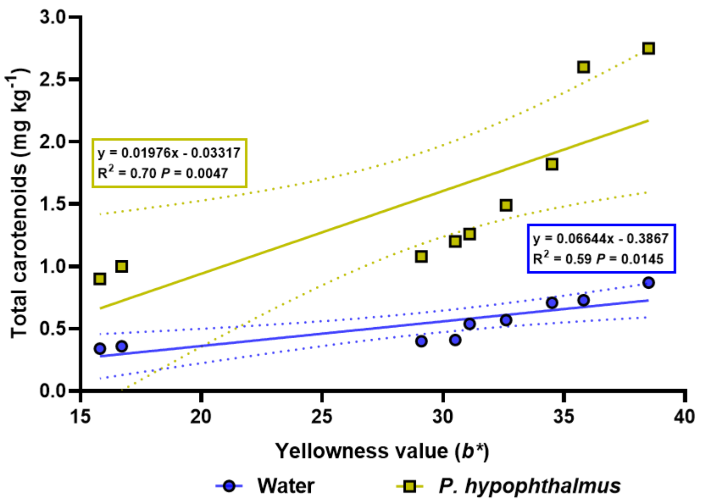

Figure 3. Linear regression curve showing the relationship between yellowness/b* values and total carotenoids in water and *P. hypophthalmus* fillets.

Phytoplankton population dynamics and species composition. The results of the descriptive analysis of plankton dynamics in the catfish cultivation pond indicate variations in ecological values for each age cluster of catfish and observation period (Table 4). The highest number of phytoplankton taxa was recorded in cluster-B, followed by cluster-A and then cluster-C. Based on the interpretation of the Shannon-Wiener diversity index, it can be concluded that the obtained species diversity levels are in the moderate category and tend to be stable in each cluster and observation period. This is because the water source utilized is the same for each pond, indicating that the observed plankton community dynamics are not significantly influenced by external factors. The abundance distribution of each phytoplankton species obtained during the study refers to six important groups: Bacillariophyceae, Cyanophyceae, Chlorophyceae, Cryptophyceae, Dinophyceae, and Euglenophyceae (Figure 5). Bacillariophyceae appears abundant and diverse in the first week for all age clusters of catfish and gradually decreases towards the end of the observation period. Cyanophyceae and Chlorophyceae groups remain relatively stable in terms of abundance and species presence in each age cluster and observation period.

Table 4

Phytoplankton population dynamics and ecological parameters in the pond of *P. hypophthalmus* in different observation periods and age groups

Parameters	*Cluster-A (Period)*			*Cluster-B (Period)*			*Cluster-C (Period)*		
	I	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
Taxa (N)	16	17	15	21	12	18	13	11	11
Diversity	0.77	1.45	1.52	1.35	1.82	1.66	0.48	0.84	1.58
Evenness	0.28	0.51	0.56	0.44	0.73	0.58	0.19	0.35	0.66
Dominance	0.69	0.32	0.32	0.30	0.23	0.31	0.81	0.47	0.26

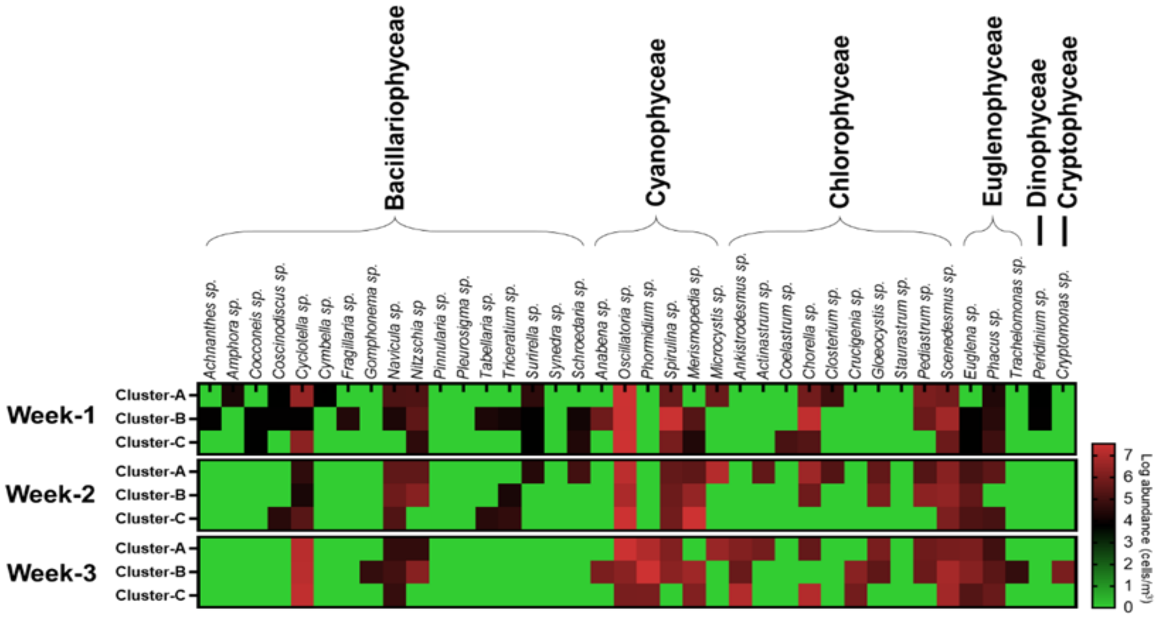

Figure 5. Heatmap of phytoplankton abundance and species composition of representative groups in different observation periods (week) and age groups (cluster).

Interrelationships between abiotic and biotic factors in supporting yellow discoloration of P. hypophthalmus fillets. The results of the Pearson correlation analysis were conducted on the compiled data from each catfish age cluster and observation period (Figure 6). Abiotic factors, including temperature, pH, redox potential, TDS, oxygen levels, ammonia, nitrite, and nitrate, were correlated with biotic factors such as total carotenoids in catfish flesh and water, the abundance of Bacillariophyceae, Chlorophyceae, Cyanophyceae, phytoplankton diversity index, and color values (L, a*, b*). The correlation analysis revealed significant relationships (r > 0.5) between various abiotic and biotic factors. Notably, temperature showed strong correlations with total carotenoids in both water and catfish flesh, indicating a pivotal role in carotenoid presence. pH exhibited significant correlations with phytoplankton diversity indices (H'), Bacillariophyceae, and Cyanophyceae. Additionally, redox potential correlated with color values (L*, a*), and TDS correlated with color values (L, a*). Oxygen levels showed a correlation with color value (a*), while ammonia levels correlated with color value (a*), phytoplankton diversity indices (H'), and Bacillariophyceae. Nitrate levels correlated with color values (L, a*), and nitrite levels correlated with color values (L, a*). Furthermore, the correlation between biotic factors revealed that the b* value, representing yellowness, exhibited a significant correlation with phytoplankton diversity index (H'). Principal Component Analysis (PCA) focused on the Cyanophyceae group, demonstrating that variations in total carotenoids (water, flesh) were closely related to the abundance variations of *Phormidium* sp. and *Anabaena* sp. (Figure 7). These cyanobacterial species appear to play a crucial role in influencing the yellowness of catfish flesh, indicating a potential ecological factor contributing to color changes that warrants further investigation.

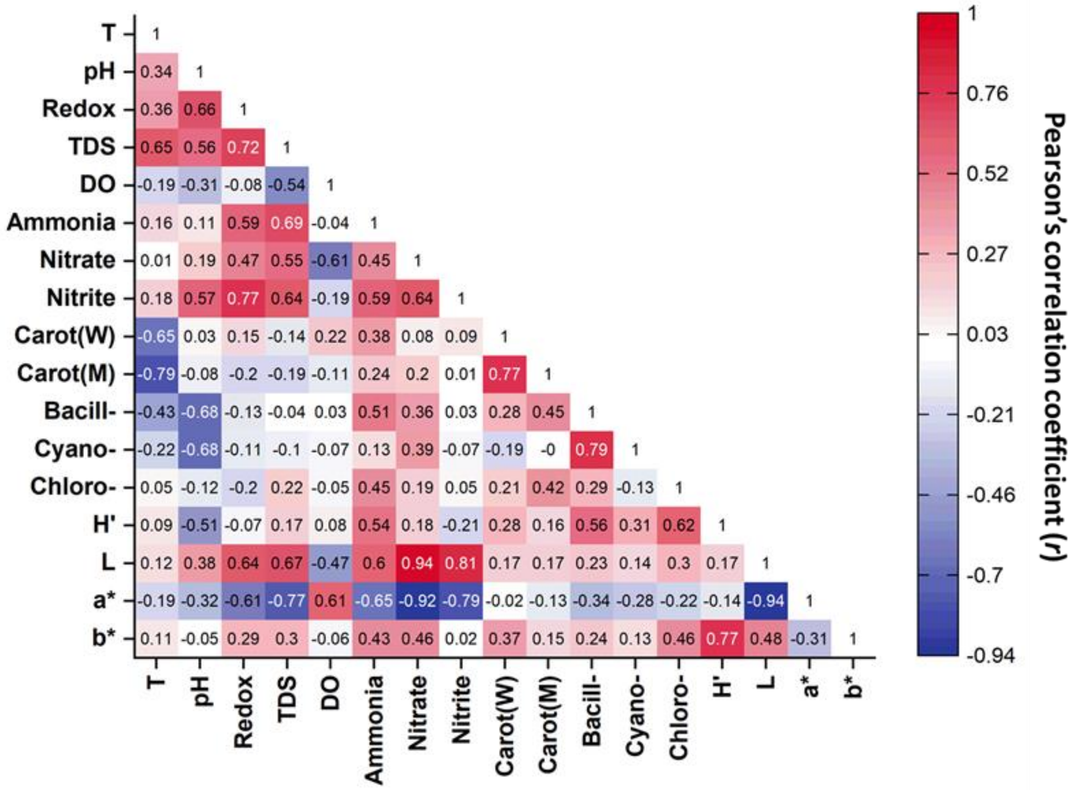

Figure 6. Matrix plot of Pearson correlation coefficients (r) between abiotic factors (T = temperature, pH, redox potential, TDS = total dissolved solids, DO = dissolved oxygen, ammonia, nitrate, nitrite) and biotic factors (Carot(W) = total carotenoid in water, Carot(M) = total carotenoid in catfish fillets, Bacill- = abundance of Bacillariophyceae, Cyano- = abundance of Cyanophyceae, Chloro- = abundance of Chlorophyceae, H' = Shannon-Wiener diversity index, L = brightness, a* = redness, b* = yellowness).

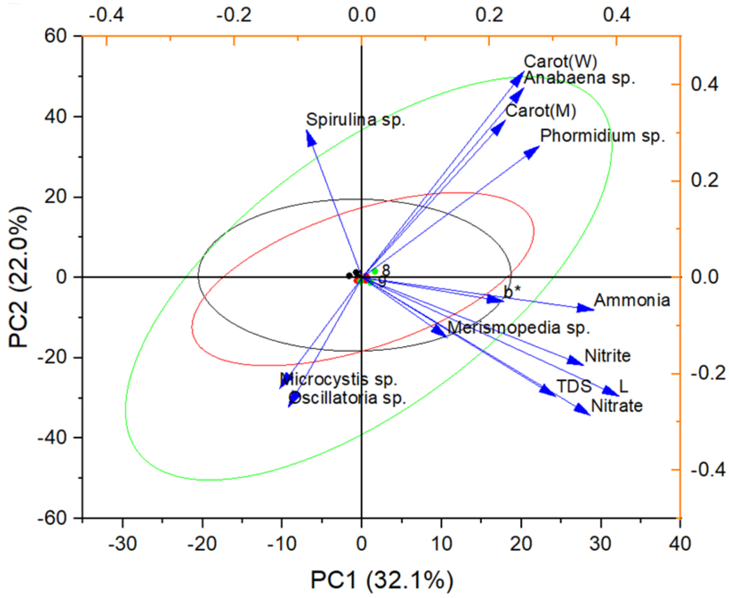

Figure 7. Biplot of principal component analysis (PCA) showing relationship between the environmental variables and fillet (color, carotenoids) indicators of *P. hypophthalmus*. The two principal components (PC1 and PC2) explained 54.1% of total variation.

Discussion. The water temperature in the catfish farming pond is considered optimal for the growth of *P. hypophthalmus*. According to Azimuddin et al (1999), the ideal temperature range for the growth of *P. hypophthalmus*, an endemic catfish species typical of the Southeast Asian tropical region, is between 28 to 32°C. Most fish species are ectothermic organisms highly influenced by environmental temperature physiologically. Temperature affects the metabolic rate, movement balance, and natural behaviors such as swimming and feeding. It also influences the fish's ability to acquire food and how they process food through digestion, nutrient absorption in the digestive tract, and energy storage. However, the influence of temperature on fish is species-specific due to the significant diversity in habitat, feeding habits, and anatomical and physiological features among different fish species. The impact of temperature depends on the timing, intensity, and duration of exposure, as well as the rate of temperature change. While short-term extreme temperature variations may have detrimental effects on fish physiology, gradual long-term variations can lead to acclimatization, such as changes in metabolic enzyme and digestive enzyme profiles (Volkoff & Rønnestad 2020).

Acidity level or pH is a value indicating the concentration of proton ions (H^+) in water. The pH values observed during the study were stable and within the normal range for *P. hypophthalmus* aquaculture. Munisa et al (2015) suggested that pH values ranging from 6.5 to 9.0 are optimal for catfish farming. Abedin et al (2017) also reported that pH conditions between 6.9 to 7.4 are stable for catfish farming across different age groups (1 to 8 months) and pond ages (5 to 10 years) in Bangladesh. The dynamics of pH values in water are generally influenced by carbon dioxide (CO_2) levels and ions reaching equilibrium. At higher temperatures, fish tend to be more sensitive to pH changes. Redox potential (E_H) is one of the crucial parameters in determining the level of oxidation and reduction processes occurring in water. Redox potential indicates the electrochemical reaction or electron availability in the system. In aquaculture systems using ponds/tanks and under aerobic conditions, the ideal EH value is above 500 mV at the water-sediment interface. In freshwater with a pH around 7.0, a DO level of 8 mg L^{-1}, and a temperature of 25°C, the E_H value is around 800 mV (Boyd 2016). Therefore, redox potential also indicates the anaerobic conditions of a pond, decreasing over time and potentially affecting the transformation by dominant microorganisms on organic compounds, toxin production, and mineral solubility (Avnimelech et al 2004).

Chien (1989) classified sediment conditions in aquaculture ponds into four categories: oxidation (400 - 700 mV), medium reduction (100 - 400 mV), reduction/

anaerobic (-100 - 100 mV), and high reduction (-300 - -100 mV). Referring to these theories, the water conditions in the *P. hypophthalmus* pond are considered anaerobic, with an EH value reaching only 75 mV and potentially being suboptimal. For comparison, the EH value obtained from other study on *P. hypophthalmus* pond, is 226.1 mV and still considered aerobic (Said & Sadi 2019). This condition may induce oxidative stress on the survival of *P. hypophthalmus* during cultivation and could be considered a recommendation for future improvements. TDS is a value indicating the concentration of inorganic and organic charges dissolved in water. TDS values are also essential parameters for assessing the suitability of habitat or water for cultivated organisms. Based on the results obtained, there was an increase in TDS values until the end of the observation, particularly in the heaviest cluster (Cluster-C), ranging from 826 to 2248 mg L^{-1}. According to the Indonesian Government Regulation No. 22 of 2021, Attachment VI regarding National Water Quality Standards, the use of water sources for aquaculture is set not to exceed 1000 mg L^{-1}. Additionally, James (2000) stated that TDS values ≤ 400 mg L^{-1} in water bodies or aquaculture ponds are still suitable for use. Referring to these regulations, the conditions in the *P. hypophthalmus* pond are not ideal or only approachable, particularly when focusing on the cluster-A pond considering the smallest size dimensions. The same stocking density in each pond with different size dimensions (cluster-A, B, C) could also potentially contribute to the high TDS values caused by the physical activities among individuals, which tend to circulate the pond's water and sediment interface.

DO is a critical factor in aquaculture as it is directly related to growth, productivity, consumption rate, and metabolism. Regular DO supply is necessary for all types of aquatic organisms derived from diffusion at the water-atmosphere interface and photosynthesis activities by phytoplankton (Liqoarobby et al 2021). In fish farming ponds, the amount of DO needed must be maintained through scientific pond management to achieve high fish production results. The observed DO value remained constant, reaching only 1 mg L^{-1}. Low DO values were also reported by Suriyadin et al (2023) in catfish farming ponds, ranging from 0.24 to 2.78 mg L^{-1}. When related to the EH parameter, the water conditions in the *P. hypophthalmus* farming pond are already considered anaerobic. Water quality standards, as explained in Regulation No. 22 of 2021, require pond conditions with a minimum DO value of 3 mg L^{-1}. Furthermore, Abedin et al (2017) recommended a minimum limit for DO values of 3.5 mg L^{-1} as the ideal condition for catfish. Although it may seem less optimal, the tolerance of catfish to low-oxygen water areas is relatively high, supported by the presence of air-breathing organs that allow catfish to stay out of the water for an extended period (Papuc et al 2019).

Nitrogen-based organic materials observed in this study are represented by ammonia (NH_3), nitrate (NO_3), and nitrite (NO_2). According to the Indonesian National Standard (SNI 01-6483.3-2000), the recommended values for ammonia and nitrite are 0.02 and 1 mg L^{-1}, respectively. Meanwhile, Regulation No. 22 of 2021 stipulates the maximum tolerance limits for ammonia, nitrate, and nitrite in Class 2 water quality, set at 0.2, 10, and 0.06 mg L^{-1}, respectively. The results indicate that the water conditions in the catfish farming pond are considered polluted and may have negative effects on *P. hypophthalmus*. The availability of excess feed and organic waste as a source or substrate for N can influence the levels of ammonia, nitrite, and nitrate in water. Nitrification is a process carried out by two groups of bacteria in oxidizing ammonia to nitrate. Ammonia-oxidizing bacteria first oxidize ammonia to hydroxylamine and then to nitrite. Nitrite-oxidizing bacteria then oxidize nitrite to nitrate, requiring 4.57 mg O_2 per mg of N to facilitate the oxidation process. Additionally, nitrification is highly dependent on environmental temperature, with an optimum range of 25 to 30^{o}C for the growth of these bacterial groups (Kim et al 2021).

In this study, the amount of nitrite was lower than the amount of nitrate, indicating the presence of other nitrite sources that have not been identified as substrates for nitrate formation. High population density, water quality dynamics, excessive feed application, or other organic or inorganic substances arising from poor management can increase the vulnerability of catfish to stress and disease. Noninfectious diseases, including environmental factors, nutrition, and genetics, can also reduce aquaculture health. Environmental diseases arise due to low oxygen, high ammonia, high nitrite, or the presence of toxins in the aquatic environment (Lisachov et al 2023). Parameters L, a*, and b* are indicators of the brightness or darkness of an object and color intensity within a specific spectrum that may represent the condition of catfish meat during observation, potentially influenced by various factors. The pigmentation of catfish meat is generally white and can change depending on the feed conditions and the availability of colorants throughout their life. Li et al (2007) conducted a study on the administration of carotenoids in pure form and their derivatives, namely astaxanthin, canthaxanthin, lutein, and zeaxanthin, to *Ictalurus punctatus*. The highest visual yellow color intensity scores occurred in fish given lutein, followed by zeaxanthin, astaxanthin, and canthaxanthin, while the lowest scores were observed in fish given a basic diet or beta-carotenoid. Fish accumulate additional carotenoids in muscle tissues, but the concentration of carotenoids varies in each tissue of *I. punctatus*.

Body fat deposits in fish can also affect color criteria, especially the L value, as high-fat conditions can contribute to high L values. Yi et al (2014) reported that the carotenoid content in the abdominal skin increased with the increase in fat content in the diet, reaching up to 14.9%. High correlation values were found between the yellowness level and carotenoid content in the abdominal region of *Larimichthys croceus*. Additionally, Chwatowska-Siwiecka et al (2016) added that there were significant differences in a* and b* values between male and female *Clarias gariepinus*, with the highest values obtained in females. Fish meat color is a species-specific characteristic determined by the amount of red muscle fibers and the concentration of pigments, including myoglobin, hemoglobin, and carotenoids. Based on the results of the multivariate analysis, it is evident that the variations in total carotenoid values (water, flesh) in this study are associated with variations in the abundance of *Phormidium* sp. and *Anabaena* sp., representing species from the cyanobacterial group in the case of catfish flesh yellowness. Prior information regarding the contribution of carotenoids as pigments from *Phormidium* sp. related to color changes in catfish flesh is limited and requires further investigation. However, a study on the pigment content of *Phormidium autumnale* by Rodrigues et al (2015) has documented 24 types of carotenoids, with the highest proportion obtained from beta-carotene (225 ppm), followed by lutein (118 ppm), and zeaxanthin (88 ppm). Additionally, reports on the toxicological aspects of *Phormidium autumnale* have documented several types of toxins, namely anatoxin-a, homoanatoxin-a, dihydroanatoxin-a, and dihydrohomoanatoxin-a. Blooming of *P. autumnale* is also associated with nutrient enrichment conditions, especially soluble inorganic nitrogen sources, and phosphorus concentrations below 0.01 mg L^{-1} in river ecosystems (McAllister et al 2016). In contrast, *Anabaena* sp. has long been known as a high-yielding source of carotenoid pigments (Saini et al 2018). Kłodawska et al (2019) reported that the optimum temperature for the growth of *Anabaena* sp. in accumulating the highest total carotenoid was achieved at temperatures of 23 and 30°C.

Conclusions. Physical and chemical conditions in the ponds for *P. hypophthalmus* aquaculture in Deli Serdang Regency, North Sumatra, Indonesia exhibit varying values depending on day-of-culture (DOC) and observation period. Notably, dissolved oxygen (DO) levels were remarkably low (< 1.5 mg L^{-1}), and organic matter pollution was evident based on total dissolved solids (TDS), ammonia, and nitrate levels, exceeding water quality standards for fish farming requirements. The number of taxa and abundance of phytoplankton increased until the final week of observation, with the highest abundance from Cyanophyceae (Cyanobacteria), followed by Chlorophyceae, Bacillariophyceae, and so forth. Strong correlations exist between total water carotenoid and flesh, particularly associated with the abundance of specific phytoplankton species such as *Phormidium* sp. and *Anabaena* sp. as revealed by Principal Component Analysis (PCA). Other supporting factors worthy of investigation included feed composition as the primary nutritional source which may also contribute to carotenoid deposition during pond cultivation. Although total carotenoid content significantly affects flesh yellowness, an in-depth analysis, especially of derivative compounds like astaxanthin, zeaxanthin, and xanthophyll, is crucial. Detection of these compounds can be enhanced using High-Performance Liquid Chromatography (HPLC) and more sensitive chromatographic techniques. Critical stages related to flesh condition were observed in cluster-B or 100 < DOC < 200 days, demanding special attention from cultivators to prevent transition to cluster-C. High ammonia levels require specific attention as an abiotic factor limiting cyanobacterial growth, especially *Anabaena* and *Phormidium*, which significantly correlated with the degree of yellowness in each cultivation cluster. Finally, post-harvest yellowness monitoring is necessary for additional information and specific guidance regarding the use of chemicals or specific treatments to mitigate yellowness impacts.

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